Verbundprojekt: Indonesien:
Korallenriff Ökosysteme und Ressourcen

Förderkennzeichen 03F0390

SCHLUSSBERICHT

Zuwendungsempfänger: Zentrum für Marine Tropenökologie	Förderkennzeichen: **03F0390A**
Vorhabensbezeichnung: **TP1.1, 1.2 und 2 (UNHAS-ZMT):** **Ökologische Grundlagen für die Ausweisung von Marinen Schutzgebieten und Ökotourismus**	
Laufzeit des Vorhabens: 01.09.2003 bis 30.06.2007	
Berichtszeitraum: 2003-2007 (Schlussbericht)	

I. Kurzdarstellung

1. Aufgabenstellung

In diesem Verbundprojekt ging es um die interdisziplinäre Erfoschung der Küstenökosysteme in einem der diversesten aber auch bedrohtesten Korallenriffregionen Indonesiens, dem Spermonde Archipel in Süd-Sulawesi. Hier sind die Riffe und angrenzende Seegraswiesen durch vielfältige Eingriffe seit vielen Jahren auf dem Rückzug: langfristige Übernutzung durch einen wachsende Bevölkerung, Einsatz von destruktiven Fischereimethoden (Bomben- und Giftfischerei), Verschmutzung der Küstengewässer durch Einleitungen ungeklärter Abwässer und Eintrag von Sedimenten tragen maßgeblich zu diesem Rückgang bei. Aufgabe dieses Vorhabens war es, die ökologischen Grundlagen für die Schaffung von marinen Schutzgebieten und Förderung des Ökotourismus zu erforschen und durch meeresbiologische Grundlagenforschung alternative Nutzungsformen wie die Aquakultur von Zierorganismen aufzuzeigen.

2. Voraussetzungen

Das Vorhaben wurde in enger Kooperation mit deutschen (AWI, AWI-Sylt, UFT-Uni Bremen) und indonesischen Partnern (CCRR-UNHAS) durchgeführt. Die in dem Verbund vertretene biologisch-ozeanographische, benthologische und molekulargenetische Expertise bot sehr gute Voraussetzungen für die Untersuchungen.

3. Planung und Ablauf des Vorhabens

Für das Vorhaben wurden zwei Doktorandinnen rekrutiert, die ihre Arbeit größtenteils vor Ort

verrichteten. Damit konnte eine intensive Auseinandersetzung mit den Fragestellungen des Projektes und eine enge Abstimmung bei der Planung und Durchführung des Vorhabens mit dem indonesischen Partner gewährleistet werden. Die Koordination mit den deutschen Verbundpartnern, dem zentralen SPICE Büro und dem Projektträger erfolgte von Bremen aus. Zentrales Element der Forschungen waren gemeinsam mit den Verbundpartnern durchgeführte Expeditionen im Mai 2004, März 2005 und September 2005 mit einem zu einem Forschungskutter umfunktionierten Tauchboot. Darüberhinaus wurden Dauerstationen eingerichtet an küstennah, in Schelfmitte und küstenfernen Riffen, um die räumliche Auflösung der Expeditionen durch monatliche Messungen der Kernparameter um eine zeitliche zu ergänzen.

Trotz bürokratischer Hemmnisse bei der Entzollung und Bereitstellung der Geräte in Jakarta, konnten durch die Hilfe der indonesischen Partner an CCRR-UNHAS die für die Arbeiten notwendigen Geräte bereitgestellt und eingesetzt werden.

4. wissenschaftlicher und technischer Stand, an den angeknüpft wurde

Für die Untersuchungen konnte auf den aktuellen Stand riffökologischer Forschung angeknüpft werden. CCRR-UNHAS blickt auf über 20 Jahre Forschungserfahrung im Spermonde Archipel zurück und ist federführend für die Durchführung der Korallen- und Fischgemeinschaftsuntersuchungen. Das ZMT konnte methodisch und technisch an wichtige Vorläuferprojekte im Roten Meer anknüpfen (FKZ 03F0151A, 03F0245A/9, 03F0356A).

5. Zusammenarbeit mit anderen Stellen

Sowohl thematisch als auch regional ergab sich eine enge Zusammenarbeit mit sozio-ökonomischen Wissenschaftlern an der UNHAS. Sie mündete unter Einbindung deutscher Partner in ein neues Verbundvorhaben (Cluster 6) ein. Durch die CCRR-UNHAS ergab sich eine enge Zusammenarbeit mit den für das Küstenzonenmanagement zuständigen Stellen. Der indonesische Partner sicherte zudem eine enge Anbindung an die nationalen und internationalen Programme, nicht zuletzt durch die Personalunion des CCRR Direktors, Dr. Jompa, als indonesischer wissenschaftlicher Koordinator des Verbundvorhabens und Executive Manager von COREMAP. Ergebnisse aus dem Verbundvorhaben wurden auch auf der gemeinsamen LOICZ-SPICE Konferenz in Bali vorgestellt.

II. Eingehende Darstellung

1. Verwendung der Zuwendung

Die Zuwendung wurde entsprechend der formulierten Projektziele für ökologische Grundlagenforschung zu (A) Ökologie und Physiologie von Korallen und (B) ihrer potentiellen Nutzung durch alternative Nutzungsformen wie der Kultur von Zierorganismen ausgegeben.

Die Ergebnisse dieser beiden im Rahmen zweier Dissertationen untersuchten Schwerpunkte werden nachfolgend im Detail dargestellt.

A. Ecology and Physiology of Corals

Photophysiological stability of the scleractinian holobiont to biotic and abiotic disturbances

Esther Borell

1. Effects of heterotrophy on bleaching susceptibility of scleractinian corals – experimental investigations

1.1 Introduction

Coral bleaching, the dissociation of corals and their zooxanthellae (endosymbiotic dinoflagellates of the genus *Symbiodinium*) in response to thermal stress is a global threat to coral reefs. Coral responses to elevated temperatures are however not uniform but often display high variation patterns of bleaching severity, differential mortality and subsequent recovery not only between different species but also among and within conspecific colonies in the same reefal habitat. Recent advances in chlorophyll fluorescence and molecular techniques have led to several lines of evidence which implicate coral bleaching with chronic photoinhibition of photosynthesis and the subsequent increase in reactive oxygen species (ROS). ROS not only cause significant damage to various cellular components of the coral-algal complex but also inhibit protein synthesis and repair. Both zooxanthellae and coral host however feature elaborate protective mechanisms against ROS, including antioxidant enzymes, heat shock proteins and certain mycosporine-like amino acids (MAA) In addition corals are able to regulate excess excitation energy via photoprotective non-photochemical quenching (NPQ) processes, which provide rapid protection against excess irradiation without the high metabolic costs associated with increased rates of the synthesis of antioxidant defences.

1.2 Research objectives

The inherent multi-trophic feeding ecology of corals may thus present an important, yet so far overlooked, factor in explaining different bleaching susceptibilities not only between species but also between individual colonies within a given species. The rationale of this study was to test the hypothesis that heterotrophic nutrient input enhances coral resilience, both directly as important nutritional asset and indirectly, by sustaining zooxanthellae photosynthesis under thermal stress. Employing pulse-amplitude-modulated (PAM) fluorometry, I experimentally investigated the effect of zooplankton feeding 1) on the functional state of the photosynthetic apparatus of zooxanthellae in the coral *Stylophora pistillata*, a thermally sensitive species, during temperature stress over time and 2) on the photosynthetic oxygen evolution and tissue concentrations of proteins and lipids of the *S. pistillata* and the thermotolerant species *Galaxea fascicularis.* All experiments comprised two treatments: starvation, i.e. corals was deprived of any particular organic material, and 2) feeding, i.e. corals were fed daily with zooplankton. This study was carried out at Hasanuddin University Marine Field Station on Barang Lompo island (05^{o} 03' S, 119^{o} 19' E; Spermonde Archipelago, southwest Sulawesi) during the dry season (July-August 2005).

1.3 Results

1.3.1 Experiment 1

Zooplankton feeding sustains photosynthesis of symbiotic dinoflagellates in the scleractinian coral *Stylophora pistillata* (Esper) during thermal stress

(re-submitted to Oecologia)

Summary
The results of the fluorescence data show that the feeding regime of *Stylophora pistillata* led to significant changes in the symbiont photophysiology over 10 days of exposure to elevated temperature. Zooxanthellae in both fed and starved corals displayed a decrease in daily maximum potential quantum yield (F_v/F_m) of photosystem II (PSII) (Fig.1), effective quantum yield ($\Delta F/F_m'$) and relative electron transport rates (rETR) over the course of 10 days (Fig.2). However the overall loss in photosynthetic activity was significantly more pronounced for starved than for fed corals. Starved corals showed strong signs of chronic photoinhibition reflected in a significant decline in nocturnal recovery rates of PSII. This was paralleled by the progressive inability to dissipate excess excitation energy via non-photochemical quenching (NPQ). Fed corals by comparison maintained high levels of NPQ and high rates of nocturnal recovery of PSII. After 10 days of exposure to elevated temperature, the daily F_v/F_m measured between 10:00 – 11:00 was significantly higher for fed than for starved corals. Feeding treatment had no significant effect on chlorophyll a and c_2 concentrations and zooxanthellae densities but starved corals had significantly lower mitotic indices than fed corals. Collectively the results indicate that heterotrophy may play an important role in reducing the photophysiological damage of zooxanthellae that typically leads to bleaching.

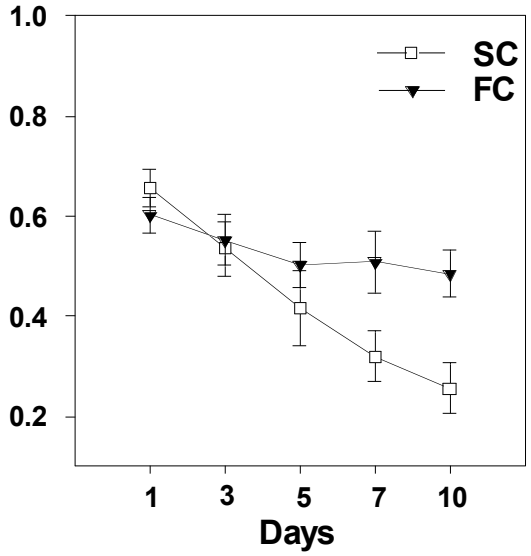


Fig. 1 Maximum potential quantum yield (F_v/F_m) of dark-adapted *Stylophora pistillata* at 31.2 ± 0.05 ^{o}C between 10:00 – 11:00 h over 10 days. Corals were exposed to either feeding (FC) or starvation (SC), (mean ± SE, n = 6).

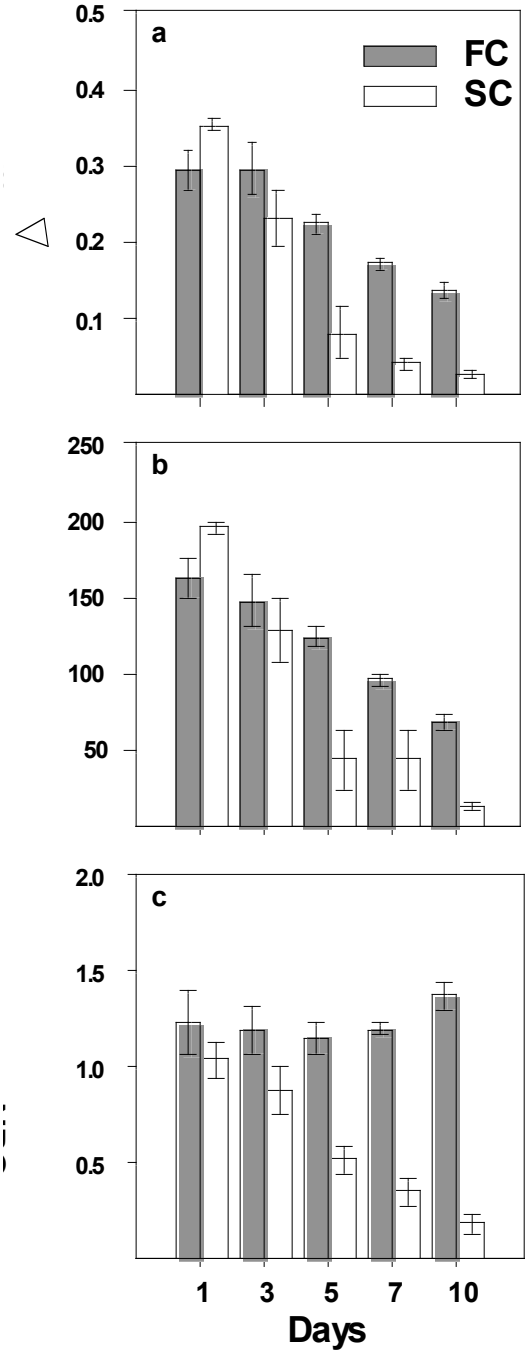


Fig. 2 Pre-dawn (05:00 – 06:00 h) measurements of (a) effective quantum yield ($\Delta F/F_m'$), (b) electron transport rate (ETR) and (c) non-photochemical quenching (NPQ) following 4 min. exposure to >1000 µmol m^{-2} s^{-1} over 10 days. Corals were subjected to either feeding (FC) or starvation (SC). Data present the average of three repeat trials (mean ± SE, n = 6).

1.3.2 Experiment 2

Differential bleaching and tissue composition of *Galaxea fascicularis* and *Stylophora pistillata* in response to zooplankton feeding under thermal stress.

(in preparation; Target Journal: Journal for Experimental Marine Biology and Ecolgy)

Summary

Feeding versus starvation had a significant effect on the gross photostnthetic oxygen production (GPP) in *Stylophora pistillata* but not in *Galaxea fascicularis* (Fig. 1). GPP decreased for *S. pistillata* of both treatments but was significantly more pronounced for starved than for fed corals (Fig.1). There was no significant change in rate of respiration for fed and starved corals of both species. The results therefore indicate that the loss in photosynthetic production in starved *S. pistillata* was not a result of increased rates of oxygen consumption but rather the consequence of photosynthetic impairment of the photosymbionts or a decrease in symbiont density or chlorophyll concentration.
Analyses of coral tissue biomass parameters revealed no significant changes in chlorophyll a and c_2 concentrations for both species regardless of treatment. As illustrated in Fig. 2 the zooxanthellae densities of starved *S. pistillata* after 15 days of exposure to elevated temperature were significantly lower than those in fed *S. pistillata* supporting the hypothesis of experiment 1 that exogeneous food may play an important role in reducing the photophysiological damage of zooxanthellae that typically leads to bleaching. This is further supported by the results for the lipid content of *S. pistillata*. Total tissue lipid content was significantly lower in starved than in fed corals (Fig. 3).
Interestingly feeding treatment also had a significant effect on the zooxanthellae densities and lipid content of *G. fascicularis* (Fig. 2 and 3) with significantly higher symbiont numbers and higher lipid concentrations in fed than in starved corals.
Previous studies have demonstrated a close link between food availability, physiological energetics, and lipid reserves in corals.
The results of this study corroborate this idea and add an important point pertaining to the mechanistic relationship between resource availability and the onset of bleaching (i.e. the loss of pigmentation). While it is now clear that the principal cause of warm water bleaching associates with the impairment of zooxanthellae photosynthesis, the ecological and physiological mechanisms underlying variation in bleaching susceptibility between and within species across reefal habitats is yet not fully understood. This study presents a strong functional correlation between resource availability of the host, the photosynthetic activity of the zooxanthellae and the susceptibility of the *S. pistillata* holobiont (coral-zooxanthellae association) to warm water bleaching. The photosynthetic activity of zooxanthellae in *G. fascicularis* by contrast were unaffected by host feeding although food was positively correlated with zooxanthellae densities and lipid concentrations. Exogenous food may be therefore of greater importance in mediating coral resilience of temperature sensitive than the more temperature tolerant species during thermal stress.
Zooxanthellae of the genus Symbiodinium comprise different genotypes with different thermal sensitivities. Furthermore host variables such as tissue thickness and polyp retraction can effect the bleaching susceptibility of different coral species. The results of this study indicate that zooxanthellae of *G. fascicularis* were more temperature tolerant than zooxanthellae of. *S. pistillata* and that a loss in photosynthetic activity due to thermal damage in *S. pistillata* may be alleviated by exogeneous food.

These findings provide useful information aiding future predictions of potential consequences of bleaching events on coral community structures, especially in coastal areas which feature characteristically high nutrient and sediment loads. Such turbid environments provide a potentially rich heterotrophic environment that may exhibit a Janus effect and actually benefit some coral species under bleaching conditions.

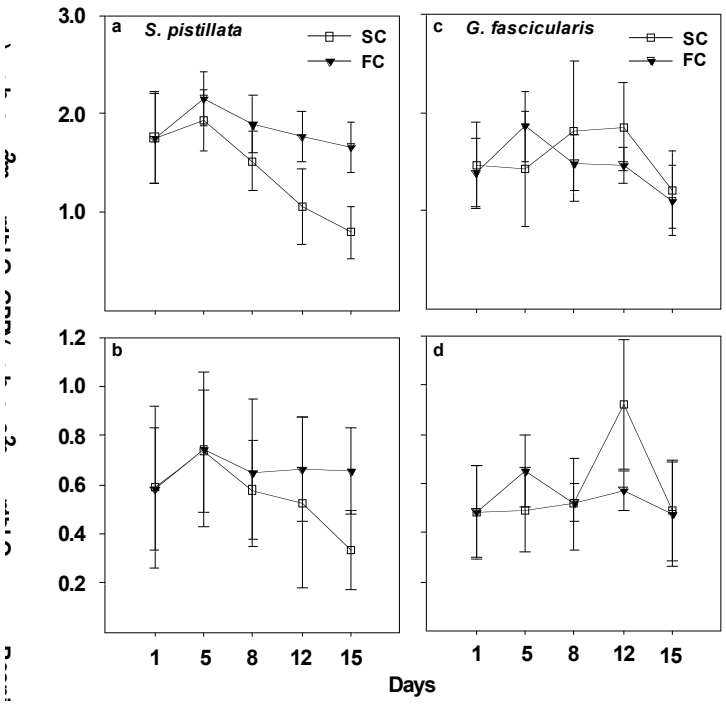


Fig. 1 Gross photosynthetic production (GPP) and respiration of *Stylophora pistillata* and (a,b) and *Galaxea fascicularis* (c,d) between 10:00 – 11:00 h at 31°C subjected to either feeding (FC) or starvation (SC) for 15 days. Data present the average of three repeat trials (95% confidence interval, n = 6).

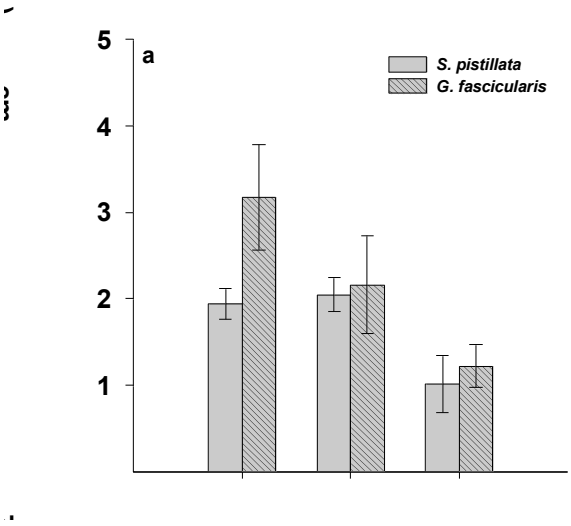


Fig. 2 a) Zooxanthellae densities (cells x 10^6 cm^{-2}) reference corals (RC) and corals after 15 days of exposure to elevated temperature and subjected to either feeding (FC) or starvation (SC) in *Stylophora pistillata* and *Galaxea fascicularis*, mean ± SE, n = 12).

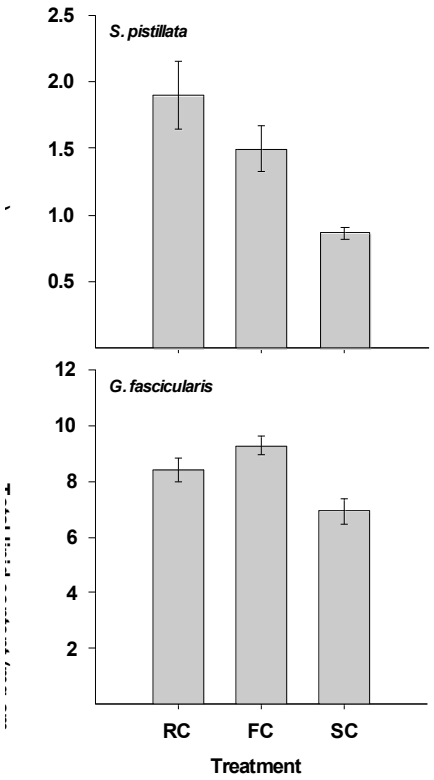


Fig. 3 Total protein content of Stylophora pistillata and Galaxea fascicularis which were frozen immediately after collection (RC) or subjected to either feeding (FC) or starvation (SC) during exposure to elevated temperature for 15 days. Data present the average of three repeat trials (mean ± SE, n = 12).

3. Supplementary study

Coral reef restauration: The effect of electrical current on coral photosynthesis

3.1 Introduction

An important objective of reef rehabilitation is to attain high levels of survival of the transplants with growth rates similar to those of the donor colonies. The mineral accretion technology as a method for coral reef rehabilitation (biorock corals) has been proposed to enhance growth and survival and increase coral fitness through enhanced metabolic efficiency of the coral transplants. Biorock corals have been claimed to grow more rapidly than natural colonies and to have greater survival rates following bleaching than coral transplants on cement. The mineral accretion technology has gained increasing popularity over the last decade and is at present a vastly utilized method in coral reef restauration projects in Indonesia. However despite its popularity there is no quantitative evidence supporting the proposed benefits of biorock corals over the utilization of other artificial substrates in reef restauration projects.

3.2 Objective

This study was designed to test the effect of seawater hydrolysis and electrical current on coral growth, mortality, photosynthetic performance and coral tissue biomass variables.

3.3 Methods

The experiment was carried out in North Sulawesi, Indonesia (01° 45' N, 125° 3' E) between April and July 2006, using the congeneric species *Acropora yongeii* and *A. pulchra* as study species. High growth rates, characteristic for the genus *Acropora* together with its high abundance in the Indo-Pacific makes this genus particularly relevant for coral reef restauration projects.

3.4 Results (submitted for the poster minisymposium at the 11th coral reef conference in Florida)

Biorock corals – backtracking the notion of enhanced growth and prosperity

Seawater electrolysis has been promoted as a mechanism to enhance growth and health of coral transplants growing on a cathode (biorock corals) but evidence supporting these claims remains largely anecdotal. This study investigates the effect of seawater hydrolysis on the maximum potential quantum yield (F_v/F_m), zooxanthellae densities, chlorophyll content and growth rates of the congeneric species *Acropora yongei* and *A. pulchra.*
Coral fragments of each species were transplanted to a depth of 5 m and grown on either 1) an iron cathode, 2) bamboo within the electric field, or 3) bamboo outside the electric field. F_v/F_m of dark adapted *A. yongei* between 10:00-11:00 h after a treatment period of 4 months was significantly higher inside and outside the electric field than on the cathode, which coincided with significantly higher zooxanthellae densities in corals inside and outside the electric field compared to corals on the cathode. By contrast there was no effect of treatment on F_v/F_m of *A. pulchra* but zooxanthellae densities were significantly higher in corals on the cathode than inside and outside the electric field.
Chlorophyll a + c_2 concentrations of both species were lowest inside the electric field.
Both species exhibited significantly higher growth rates within the electric field than on the cathode or outside the electric field.
Since calcification is linked to zooxanthellae photosynthesis the low growth rates, low F_v/F_m and concurrent low zooxanthellae densities of *A. yongei* grown on a cathode indicate that electrochemical processes through seawater hydrolysis may adversely effect important physiological processes in some coral species leading to reduced growth and health. Although growth was electrically stimulated in both species, the results of this study provide no support for the proposed physiological benefits of biorock corals over corals transplanted onto other artificial substrates.

B. Ecology and Use of Corals

Population Demographics of *Heliofungia actiniformis* (Fungiidae), a Coral Species Exploited for the Live Coral Trade in Spermonde, Indonesia

Leyla Knittweis

1. Introduction

Marine ornamental species are collected around the world to supply specimens for the international aquarium trade, which is estimated to be worth 200-330 million US$ annually. Indonesia is the world's largest exporter of live marine ornamental corals since the 1980s (Wabnitz et al. 2003) and *Heliofungia actiniformis* ranks in the top five of all coral species being exploited for the live coral market (Green and Shirley 1999). An export quota system is in place, but knowledge on the population ecology of this species is limited (Abe 1937, 1940) and altogether lacking for the Indo-Malay Archipelago.
Indeed, the information availbale on the population dynamics of scleractinian corals in general is scatterd, and particularly so for large-polyped species. This is partly due to difficulties in studying population dynamics because of the extreme slow growth and long lives of scleractinian corals (but see Connell et al., 1997; Edmunds and Elahi 2007). Furthermore the interpretation of size-frequency data using standard assessment techniques is rarely possible because of the corals' colonial nature: processes such as fragmentation, fusion and partial colony mortality distort age-size relationships in the great majority of species (Babcock 1991; Hughes 1984; Goffredo et al. 2004). In combination with variable growth rates and great longevity, this may result in a wide size range within a single cohort (Hughes and Connell 1987).
However, this is not the case for coral species which grow in distinct forms (e.g. *Pocillopora verrucosa*, Grigg 1984; *Corallium rubrum*, Tsounis et al. 2006), or indeed where partial colony mortality is evident and can be quantified readily (e.g. *Goniastrea aspera*, *Platygra sinensis*, Babcock 1991; *Siderastrea siderea*, Lewis 1997). Furthermore, a few aclonal coral species do exist, where polyps are solitary and where size and age are directly related. The Fungiidae (Dana, 1846) in particular are a family with a number of conspicuous unitary species, which lend themselves to both size and age based demographic assessments (see previous studies of *Fungia granulosa*, Chadwick-Furman et al. 2000; *Ctenactis echinata*, *Fungia scutaria*, *Fungia fungites*, *Danafungia* spp., Goffredo and Chadwick-Furman 2003).
A further aspect to be considered in the design of successful fishery management recommendations is the extent of population connectivity provided by dispersing larvae. Due to the inherent difficulty in studying coral larval dispersal pathways in situ, most information to date has come from the study of the indirect effects of larval dispersal on the genetic differentiation and gene flow among populations (e.g. Ayre and Hughes 2000; Nishikawa et al. 2003; Magalon 2005; Vollmer and Palumbi 2007). However, a number of factors have been implicated in shaping present-day genetic structure of marine populations, including the roles of (1) larval dispersal capabilities (Ayre and Hughes 2000; Lourie et al. 2005), (2) present day physical oceanographic features (Williams and Benzie 1996; Rodriguez-Lanetty and Hoegh-Guldberg 2002), and (3) historical events (Nelson et al. 2000; Barber et al. 2002).
Our aims were thus to (1) study the population dynamics of *H. actinformis* (2) examine genetic variability and genetic affinities of *H. actiniformis* populations on both a small (among reefs 7-65 km apart) and large scale (among reefs up to 2,900 km apart). The result's implications are discussed in light of potential management approaches to the exploitation of this species for the coral aquarium trade.

2. Materials and methods

2.1 Population Dynamics

Study Area

The Spermonde Archipelago in South Sulawesi extends approximately 60km offshore, consists of about 150 islands with fringing reefs, as well as a large number of barrier and submerged patch reefs (Tomascik et al. 1997), and has supported intensive fishing activity since at least the 14th century (Erdmann 1995). Today the archipelago provides livelihoods to thousands of fisher households scattered over the islands (Pet-Soede et al. 2001), and supports one of largest live coral fisheries in Indonesia (Raymekers 2001). Reef degradation is widespread, with causes including industrial and sewage pollution from nearby Makassar, extensive bomb fishing, anchor damage and over-harvesting of coral reef resources (Edinger et al. 1998).

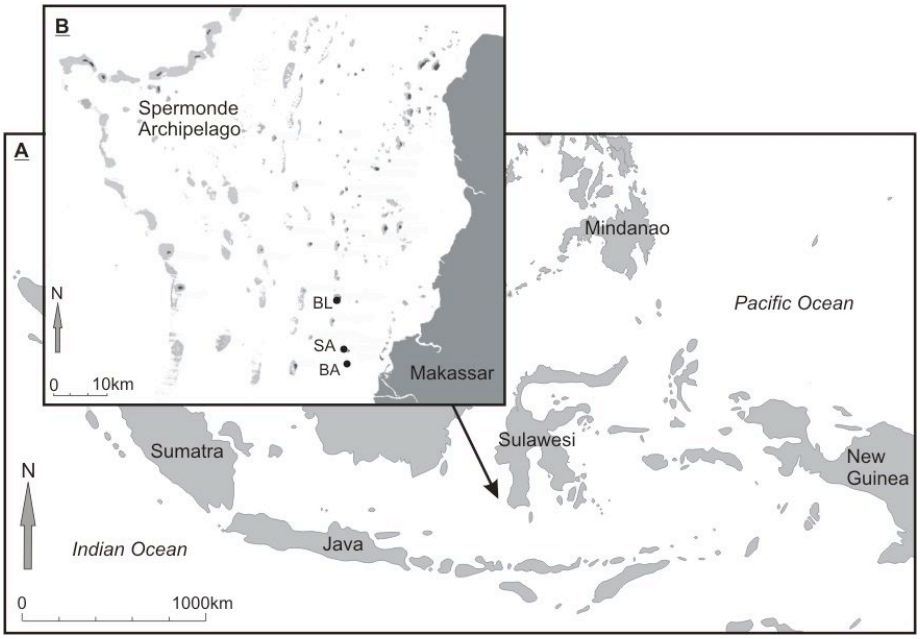


Fig. 1 A Location of Spermonde Archipelago within the Indo-Malay Archipelago **B** Sampling sites in the Spermonde Archipelago. BA: Bone Baku; SA: Samalona; BL: Barrang Lompo.

Moll (1983) identified a number of ecological reef zones based on shelf bathymetry and the distribution of coral species, with zone one located closest to the shore, and the shelf bottom dropping almost vertically to depths of over 800m (de Klerk 1983) adjacent to zone four. The present study focussed on reefs of Bone Baku (BA), Samalona (SA) and Barrang Lompo (BL), all located in zone two (Fig. 1). We deemed it necessary to survey *H. actiniformis* at three replicate sites to look into the representativeness of results: all reefs investigated during pilot surveys in October 2004 and March 2005 differed in levels of rubble cover, and hence presumably in habitat stability / exposure to disturbances for mushroom coral polyps. An extensive survey of local fishermen confirmed that *H. actiniformis* harvesting for the live coral trade does not take place at any of these sites (Knittweis & Wolff, unpublished). Thus these sites are considered representative for unfished, natural populations of this species.
Spermonde has monsoonal weather with two major seasons, the wet north-western monsoon and dry south-eastern monsoon, which extend from June-September and December-March respectively (Tomascik et al. 1997). We surveyed the north-western sides of the chosen reefs, which are exposed to the stronger hydrodynamic regimes of the rainy season, to ensure all potential life history phases were represented.

Polyp Growth

The growth rates of 82 polyps were measured in Barrang Lompo at 10-12m depth from June 2005 till December 2006. Corals were selected to cover as wide a size range as possible (3 to 181 mm length), which included polyps in the anthocaulus phase, and stained in situ using the alizarin dye technique (Lamberts 1978). At the end of the growth interval stained corals were collected, tissue removed with a brush, and growth after staining measured in situ. For those polyps where only very little growth had taken place, small fragments of skeleton were removed at the polyp edge, and measured ex situ. All measurements were done by the same observer using calipers at 6 equally spaced points around the polyp perimeter.
To establish the relationships between polyp length, width and skeletal mass, 85 detached, solitary polyps from reef slopes around Barrang Lompo Island were collected in July / August 2005, cleaned of any visible sediment / epifauna, measured and weighed using the buoyant weight technique (after Jokiel et al. 1978) before being replaced. 72 attached polyps were additionally measured in situ to establish the relationship between polyp length and width for the smaller size classes.
Growth was expressed according to the von Bertalanffy growth model $L_t = L_\infty (1-e^{-kt})$, where L_t=polyp length at age t, L_∞= asymptotic length, k= annual growth rate constant, and t= polyp age in years (von Bertalanffy, 1938). A Gulland and Holt plot was used to visualize all growth data and to show growth increments as related to mean size during the growth interval. For the calculation of the growth parameters k and L_∞, a Munro Plot was used: the asymptotic length was computed by repeating the corresponding calculations of k for each individual until the coefficient of variation between the single k-values was lowest (Munro 1982). The nine largest polyps were excluded from the calculations to avoid a disproportionate influence on k by polyps approaching L_∞.
A growth performance index (Φ) was calculated compare the results of the present study with those of other mushroom coral species obtained by previous authors. The index was computed as $\Phi = 2\log L_\infty + \log k$ (Munro and Pauly 1983).

Population size and age structure

In April / May 2005 *H. actiniformis* size-frequency distributions were obtained from belt transects at the three chosen sites. Transects were 150m long and 6m wide and set up parallel to the shore. Since an initial pilot survey revealed spatially clustered populations of *H. actiniformis*, transects were only set after encountering a minimum of 5 polyps in a 3m by 6m area. Two transect lines were set at each site, one at 8 and one at 12m, effectively sampling a depth range of 7-15m depending on reef-slope inclination, which is where *H. actiniformis* populations were concentrated. Within each transect, all *H. actiniformis* polyps were examined and their length (along the mouth axis, after Abe 1940) as well as life cycle stage recorded as (1) attached polyp on reef anthocaulus (2) attached polyp on adult polyp or (3) detached anthocyathus. Transects were in addition divided into 30 plots measuring 5x6m, in which the presence/absence of rubble and sandy patches with rubble pieces was recorded.
For the purpose of data analysis, data from 8 and 12m was combined at each site and size-frequency distributions per $500m^2$ subsequently calculated. The age-length relationship obtained from growth monitoring at Barrang Lompo was used to convert size-frequency data to age-frequency distributions. Differences in population age structures between sites were visualized through age histograms and the mean ages, as well as the amount of variation in polyp sizes (coefficient of variation, CV) of the distributions were calculated as proxies for possible differences in disturbance regimes. The shapes of the age-frequency distributions were statistically compared between populations censused at Bone Baku, Samalona and Barrang Lompo using a two-sample Kolmogorov-Smirnov (KS) test.
Total mortality rates (Z) were calculated separately for the attached and free-living life cycle phases by applying a length-converted catch curve analysis to the length-frequency data (Pauly, 1984) using the growth parameters k and L_∞ obtained in this study. Polyps sized 0-4 and 4-15cm in length were thus analysed separately. Individuals exceeding 15cm in length were excluded from the analysis to eliminate inaccuracies in the relationship of length and

age in larger polyps (after Sparre and Venema 1998). The significance of the differences between sample regression coefficients was tested by two-tailed student's t-tests.

Cohort biomass and potential yield

H. actiniformis populations were described by the application of a modified version of the Beverton and Holt (1957) yield per recruit model, which allowed us to estimate the age at maximum production of a year class, and thus the optimal size and age for harvest. For this purpose an age-specific cohort biomass curve was generated using the polyp growth curve and the average of the natural mortality rates calculated above (after Grigg, 1984; Chadwick-Furman et al. 2000; Tsounis et al. 2007).

2.2 Genetics

Collection of samples

Heliofungia actiniformis tentacle clippings were collected from a total of ten sites throughout the Indo-Malay Archipelago (Fig. …). Nine locations were in Indonesia: Adi, Gilli Trawangan, Komodo, Manado, Pulau Seribu, Saboeda, Pulau Sembilan, Spermonde Archipelago, Tilamuta and one in the Philippines: Cebu. In the Spermonde Archipelago samples were collected from nearshore (Barrang Lompo, Samalona), midshelf (Lanyukang, Sarappokeke) and outershelf (Kapoposang) reefs. Wherever possible, tissue samples were collected from polyps at least 50 m apart and of different colour morphs to reduce the likelihood of sampling genetically identical clones. Tissues were stored in 96% ethanol at 4 °C. Variable numbers of samples collected reflect species abundance levels at sampling sites.

Extraction, PCR amplification, and sequencing of DNA

DNA was extracted with a 5% Chelex® solution (Walsh et al. 1991) and approximately 650 base pairs of the ITS1-5.8S-ITS2 rDNA region were amplified by the polymerase chain reaction (PCR) using the coral specific primer A18S (GATCGAACGGTTTAGTGAGG; Takabayashi et al. 1998a) and universal primer ITS-4 (TCCTCCGCTTATTGATATGC; White et al. 1990). The PCR reaction was carried out in a total volume of 50µl. PCR reactions contained 1 µl template DNA, 10 mM TrisHCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP-mix, 0.4 mM of each primer, 40 µg BSA (bovine serum albumin) and 1 unit *Taq* polymerase (Promega). Amplification was performed in an Eppendorf Gradient S Thermal Cycler as follows: 30 cycles of 1 min at 94°C, 2 min at 55°C, 3 min at 72°C (Rodrigues-Lanetty and Hoegh-Guldberg, 2002). Successfully amplified PCR fragments were purified using the peqGOLD Cycle Pure Kit (Safety-Line; PeqLab Biotechnology GmbH, Erlangen, Germany) following the instructions of the manufacturer. Sequencing of both strands was conducted with the PCR primers, the DyeDeoxy Terminator chemistry (PE Biosystems, Foster City, U.S.A.), and an ABI automated sequencer.

Data analysis

The obtained sequences were aligned with a sequence of *Siderastrea stellata* (Forsman et al. 2005) using ClustalW (Thompson et al. 1994) as implemented in the software Bioedit version 7.0.9 (Hall 1999) to delimit the ITS1, 5,.8S and ITS2 regions. The best fit model of nucleotide substitution was calculated by performing hierarchical likelihood ratio tests with Modeltest version 3.06 (Posada and Crandall 1998), and considered in subsequent analyses.
Inter- and intra-population genetic diversity indices, such as haplotype diversity (*h*) and nucleotide diversity (π) (Nei 1987), were calculated with the programme Arlequin version 3.11 (Excoffier et al. 2005). Population structure was further examined using Φ_{st}, an analogue of conventional F-statistics which considers both the number of haplotypes and the

number of mutations in the sequences being investigated. The analysis of molecular variance (AMOVA) as implemented in Arlequin was used to determine the proportion of genetic variation within and between populations. A hierarchical AMOVA was subsequently carried out to look into potential population groupings according to geographic as well as oceanographic affinities.
Estimates of pairwise Φ_{st} were plotted against geographical distance and significance of the resulting correlation was determined by a Mantle test (Manly 1994) as well as reduced major axis regression, using Isolation by Distance (IBD) Web Service version 3.14 (Jensen et al. 2005; http://ibdws.sdsu.edu). Geographic distances consisted of the present-day shortest path by sea between any two populations, calculated in Google Earth version 4.0.13. Analyses had a nested design, with (1) small scale geographic variations investigated for sampling sites within the Spermonde Archipelago and (2) large scale geographic variations investigated for the Indo-Malay Archipelago.
A haplotype network based on pairwise sequence differences was created using the programme TCS version 1.21 (Clement et al. 2000). This method defines connections among haplotypes that have a cumulative probability of 95% of being true following the parsimony principle. Using these connections and the inferred missing intermediate stages, TCS plots a network. Gaps in the alignment resulting from base insertion/deletion were treated as a fifth nucleotide state.

3. Results

3.1 Population Dynamics

Polyp growth

The growth rate of coral polyps declined linearly with length (Fig. 2A), from an average of 11.5 ± 3.6 mm year^{-1} for the 15 smallest corals monitored (3-39 mm in length at the start of measurements), which were still in the attached life-cycle phase, to an average of 1.0 ± 1.1 mm year^{-1} for the 15 largest corals monitored (132-181 mm in length at the start of measurements). Averaged over the entire size range of corals investigated, the growth rate was 5.7 ± 4.5 mm year^{-1}. The Munro Plot revealed a minimal coefficient of variation for the growth constant (k) = 0.0741 and the maximum expected polyp length (L_∞) = 163mm (Fig. 2B). The value of the growth performance index (Φ') was 3.29; table 1 shows the growth performance index values estimated for mushroom corals in previous studies.

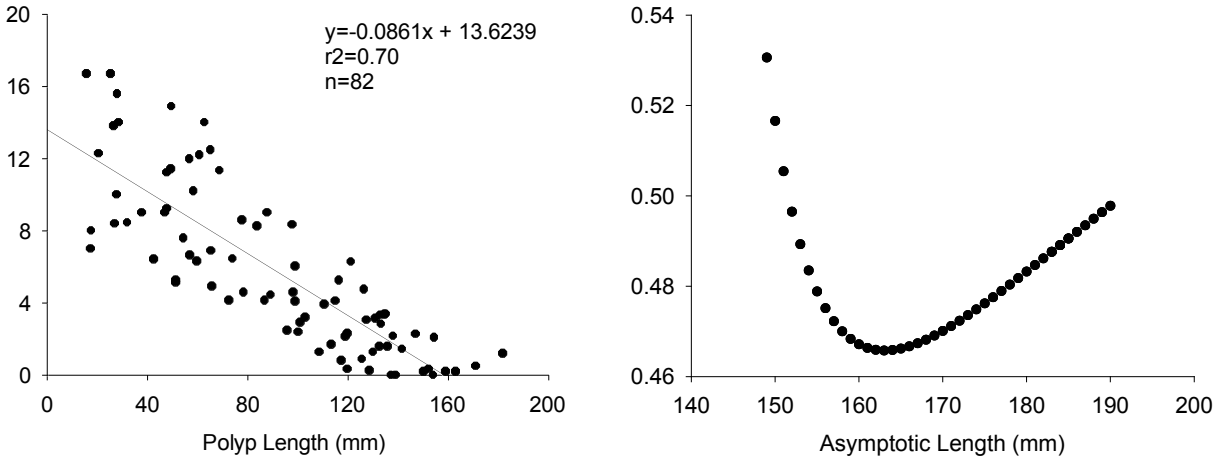


Fig. 2 A Gulland and Holt-Plot showing the linear decline in growth of *H. actiniformis* polyps with size; polyp length refers to the length attained at the mid-point between measurements.

B Coefficient of variation around average growth rate (k) for a series of asymptotic lengths (L_∞)

During the lifespan of *H. actiniformis* width varied linearly with length (Fig. 3A), i.e. polyp shape did not change over time and growth was isometric. Polyp buoyant weight increased exponentially with weight (Fig. 3B), and the equation $W = a*L^b$, could thus be used to calculate polyp biomass in subsequent analyses, where W was the coral body weight, L the coral length, b the regression line slope (i.e. the exponent of the length-weight relationship) and a the x-axis intercept (after Sparre and Venema 1998).

Table 1 Values of the growth performance index in different species of mushroom coral

Species	**Location**	**Depth**	**L_∞**	**k**	**Φ'**	**Reference**
Ctenactis echinata	Sharm el Sheik, Egypt	6-12	323	0.109	4.05	Goffredo 1995
Ctenactis echinata	Eilat, Egypt	2-40	428	0.053	3.99	Goffredo & Chadwick-Furman 2003
Fungia paumotensis	Mururoa, Tahiti	10	147	0.404	3.94	Bablet, 1985
Fungia scutaria	Eilat, Egypt	2-40	248	0.068	3.62	Goffredo & Chadwick-Furman 2003
Fungia fungites	Great Barrier Reef, Australia	0-3	133	0.215	3.58	Stephenson & Stephenson 1933[a]
Heliofungia actiniformis	Palau, Micronesia	0-2	195	0.084	3.50	Abe 1940
Fungia fungites	Eilat, Egypt	2-40	250	0.049	3.49	Goffredo & Chadwick-Furman 2003
Danafungia spp.	Eilat, Egypt	2-40	227	0.054	3.44	Goffredo & Chadwick-Furman 2003
Heliofungia actiniformis	Sulawesi, Indonesia	10-12	163	0.074	3.29	Present Study
Fungia granulosa	Eilat, Egypt	6	118	0.110	3.18	Chadwick-Furman et al. 2000
Fungia fungites	Sharm el Sheik, Egypt	6-11	107	0.088	3.00	Goffredo 1995[a]
Diasteris distorta	Okinawa, Japan	-	19	0.360	2.11	Yamashiro & Nishihira 1998

[a] referenced in: Chadwick-Furman et al. 2000

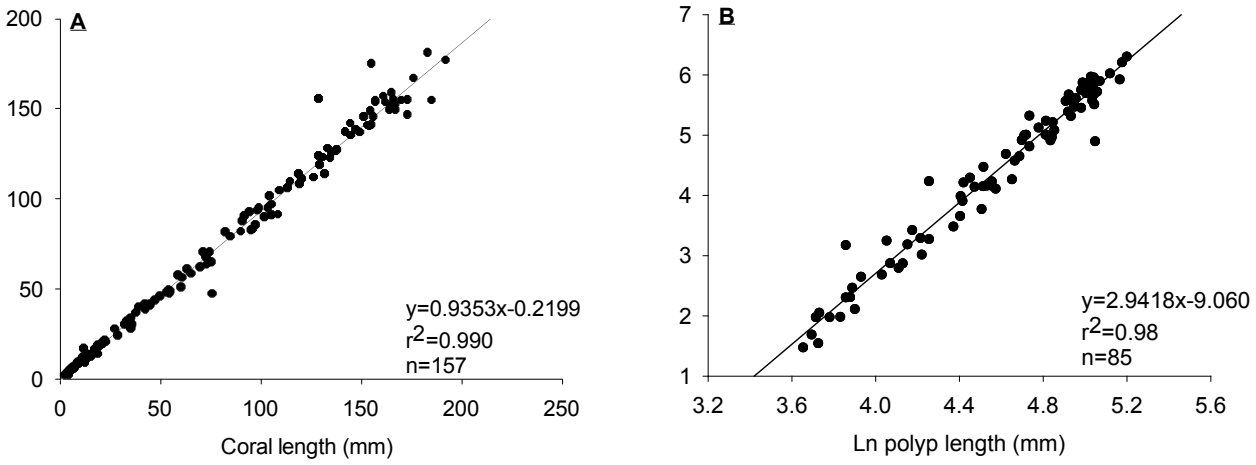


Fig. 3 A Variation of polyp width with polyp length **B** variation of polyp weight with polyp length

Population size and age structure

Age-frequency distributions of all belt transects are shown in Fig. 4 and show a similar pattern despite the differences in rubble cover (Fig. 5). Populations were dominated by young individuals with 39%, 45% and 52% of polyps belonging to the 0-4 year age class intervals at Bone Baku, Samalona and Barrang Lompo respectively. Mean polyp age was highest at Bone Baku (12.6 yrs), noticeably less at both Samalona (9.22 yrs) and lowest at Barrang Lompo (8.28 yrs). Conversely, the CV was lowest at Bone Baku (1.08) and highest at Barrang Lompo (1.17), with Samalona again in between the two (1.11). The two-sample Kolmogorov-Smirnov test showed no significant differences between the shapes of the age-frequency distributions found at Bone Bakue / Samalona and Bone Baku / Barrang Lompo (p=0.05).

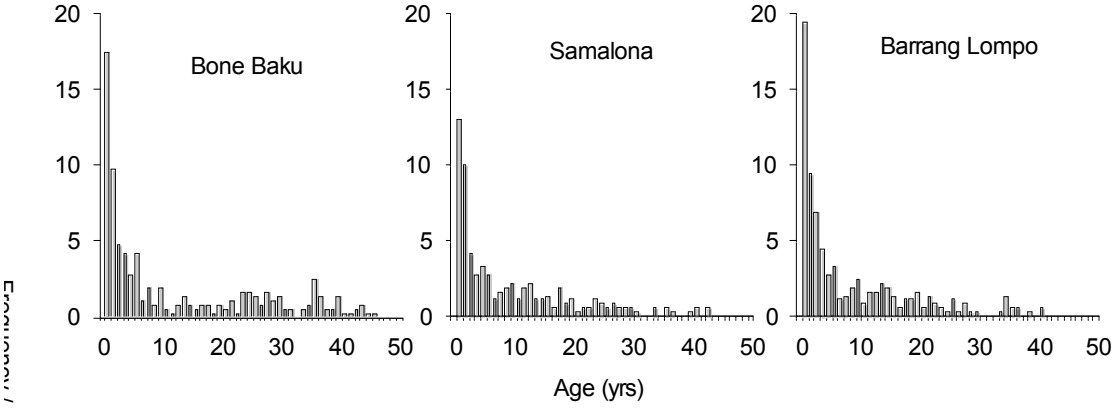


Fig 4 Age-frequency distributions of *H. actiniformis* populations at Bone Baku (n=331), Samalona (n=242) and Barrang Lompo (n=281).

Only a single adult polyp with asexual buds growing from the underside of the skeleton (n=9) was found, at Bone Baku. All other *H. actiniformis* polyps ≤ 3cm in length measured over the course of this study were found either growing on anthocaulus stalks attached to the reef (97.9%), or in the immediate vicinity of such a stalk if detached prematurely.

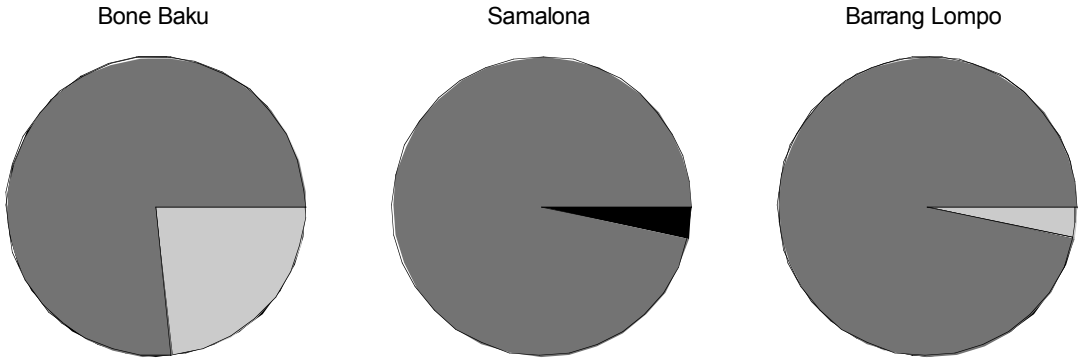


Fig. 5 Reef condition at study sites: percentage of reef segments which contained rubble (black), sandy patches with a limited number of rubble pieces (light grey) and no rubble at all (dark grey).

Anthocauli grew in clusters, with an average of 6.5 (±3.6), 5.0 (± 4.1) and 4.8 (±5.6) stalks branching out from a single sexual recruit at Bone Baku, Samalona and Barrang Lompo respectively. The maximum number of anthocauli found in a cluster was 46, recorded at 8m depth at Barrang Lompo in October 2005. During the initial transect surveys, 98.3 % of polyps sized ≥ 4cm had detached and were free living; the biggest attached polyp measured in the field was 7.5 cm long and was found at Bone Baku. The smallest detached polyps measured 2.9, 0.9 and 2.6 cm at Bone Baku, Samalona and Barrang Lompo respectively.
Far steeper slopes illustrate the higher mortality rates of the young, attached *H. actiniformis* compared to those of detached polyps (Fig. 7). The regression analyses showed high r^2 values (0.81-0.97) except for polyps 4-15cm long at Bone Baku, where less polyps sized 11-13cm and more polyps sized 13-15cm than expected with a constant mortality rate were found (r^2= 0.57).
The comparison of slopes for polyps sized 0-4cm between Bone Baku/Barrang Lompo, Bone Baku/Samalona and Barrang Lompo/Samalona revealed no significant differences (t=1.34, 0.02, 1.15 respectively, df=4, p=0.01). Similarly, the comparison of slopes for polyps sized 4-15cm between Bone Baku/Barrang Lompo, Bone Baku/Samalona and Barrang Lompo/Samalona revealed no significant differences (t=1.77, 1.48, 0.67 respectively, df=18, p=0.01).

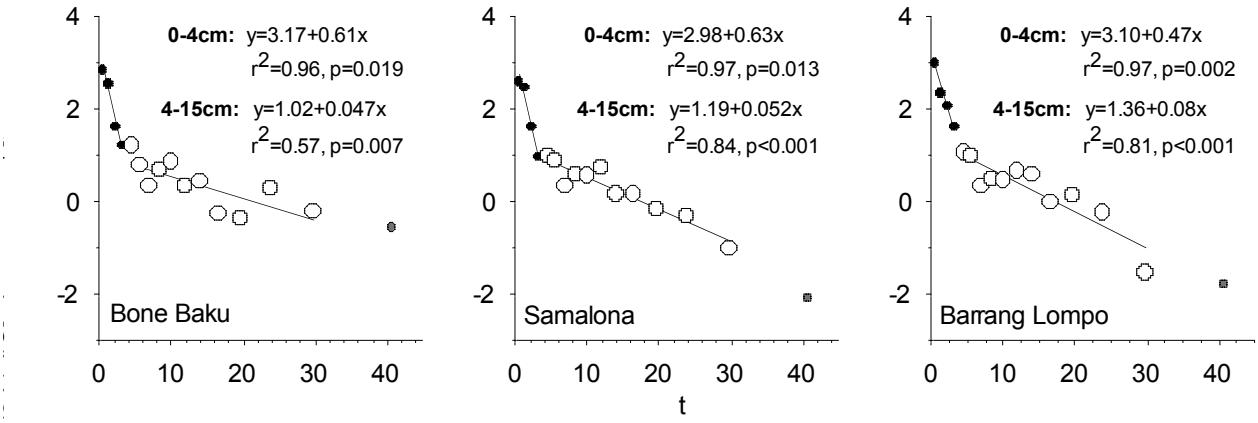


Fig. 7 Linearized catch curves based on length composition data for *H. actiniformis*. 0-4cm: black circles, 4-15cm: light grey circles, 16cm: dark grey circles.

Cohort biomass and potential yield

The biomass curve calculated for *H. actiniformis* showed a rapid increase for young polyps, before reaching the maximum of 6.54g at an age of 20years and a corresponding length of 12.6cm (Fig. 8).

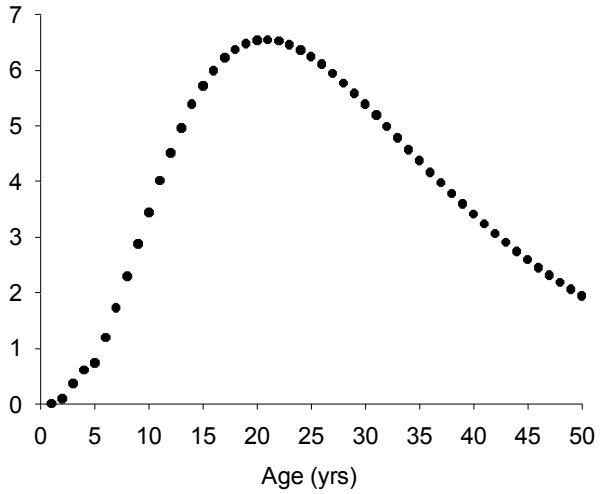


Fig. 8 Biomass per recruit curve for *H. actiniformis*.

3.2 Genetics

Sequence Analysis

In total, 21 different haplotypes were defined by 22 polymorphic sites in 173 obtained sequences. The nucleotide sequences were submitted to the European Molecular Biological Laboratory (EMBL); accession numbers are AM849550-AM849570. The best fit model of nucleotide substitution was Jukes and Cantor + I (JC+I), with estimated base frequencies and invariable sites (i) of 0.9839. Within the 623 bp alignment, the length of ITS-1 was 239 bp (complete); 5.8S and ITS-2 were 157 bp (complete) and 217 bp (partial) respectively. The full ITS-1 segment was more variable than the partial ITS-2 segment, with an average variation of 7.9% and 1.4%, respectively, whilst the 5.8S region was identical in all sequences.

Table 2 Sampling localities, sample size and genetic diversity measures ± standard deviation.

Sampling Site	Acronym	n	h	π
Adi	Ad	4	0.50 (±0.27)	0.0048 (±0.0038)
Barrang Lompo	BL	25	0.83 (±0.05)	0.0021 (±0.0015)
Cebu	Ce	30	0.68 (±0.08)	0.0025 (±0.0017)
Gilli Trawangan	Gi	9	0.69 (±0.15)	0.0025 (±0.0019)
Kapoposang	Kp	9	0.69 (±0.15)	0.0026 (±0.0019)
Komodo	Ko	8	0.82 (±0.10)	0.0036 (±0.0025)
Lanyukang	La	14	0.87 (±0.05)	0.0027 (±0.0019)
Manado	Ma	11	0.71 (±0.14)	0.0034 (±0.0023)
Pulau Seribu	PS	14	0.54 (± 0.11)	0.0015 (±0.0012)
Saboeda	Sb	5	0.40 (±0.24)	0.0020 (±0.0017)
Samalona	Sm	11	0.73 (±0.14)	0.0031 (±0.0022)
Sarapokeke	Sr	10	0.80 (±0.09)	0.0032 (±0.0022)
Pulau Sembilan	Se	11	0.78 (±0.12)	0.0032 (±0.0022)
Tilamuta	Ti	11	0.65 (±0.11)	0.0009 (±0.0009)

Table 3 Analysis of Molecular Variance (AMOVA) results: Spermonde Archipelago. Φ_{st} = 0.093, *p* = 0.022.

Source of Variation	Degrees of Freedom	Sum of squares	Variance component	% Variation
Among populations	4	7.680	0.08372	9.30
Within populations	64	52.262	0.81659	90.70
Total	68	59.943	0.90031	

Table 4 Matrix of pairwise population Φ_{st} values, Spermonde Archipelago. Sm: Samalona; BL: Barrang Lompo; Sr: Sarappokeke; La: Lanyukang; Kp: Kapoposang

	Sa	Bl	Sr	La	Kp
Sa	0.000				
Bl	0.070	0.000			
Sr	0.111	-0.006	0.000		
La	0.220**	0.075	0.062	0.000	
Kp	0.302**	0.190*	0.004	-0.042	0.000

* p<0.05, ** p<0.01

Genetic Diversity Measures

Haplotype diversity (*h*) within populations ranged from 0.40 at Saboeda to 0.87 at Lanyukang in the Spermonde Archipelago. Average nucleotide diversity (π) ranged from 0.0009 at Tilamuta to 0.0048 at Adi. Levels of haplotype and nucleotide diversity were not always correlated, the most striking example being Adi in New Guinea, which had one of the lowest haplotype diversities (*h* = 0.5), yet the highest nucleotide diversity (Table 2).

Table 5 Analysis of Molecular Variance (AMOVA) results: Indo-Malay Archipelago. Φ_{st} = 0.26, p = <0.0001

Source of Variation	Degrees of Freedom	Sum of squares	Variance component	% Variation
Among populations	13	53.895	0.27809	25.92
Within populations	159	126.379	0.79484	74.08
Total	172	180.274	1.07293	

Population Subdivision

Weak but nonetheless significant genetic structuring was found within the Spermonde Archipelago (Φ_{st} = 0.093, p <0.02; Table 3). The pairwise Φ_{st} values revealed the largest genetic subdivision to be present between the site closest to the shore (Samalona) and the sites at the shelf-edge (Kapoposang, Lanyukang). A significant genetic structure was also found between the populations in Barrang Lompo and Kapoposang (Table 4).
AMOVA showed high levels of genetic structuring in the Indo-Malay Archipelago (Φ_{st} = 0.26, p <0.0001; Table 5), indicating restricted gene flow between the populations investigated. Grouping the populations into the five groups (1) Adi (2) Barrang Lompo, Cebu, Gilli Trawangan, Manado, Kapoposang, Komodo, Lanyukang, Samalona, Sarappokeke, Pulau Sembilan (3) Pulau Seribu (4) Saboeda (5) Tilamuta, explained the maximum of the variation among the populations (Φ_{ct} = 0.32, p = 0.007; Table 6).

Isolation by distance analyses (Fig. 9) revealed a significant increase in genetic diversity with increasing geographic distance on the small scale within the Spermonde Archipelago as well as across the Indo-Malay Archipelago.

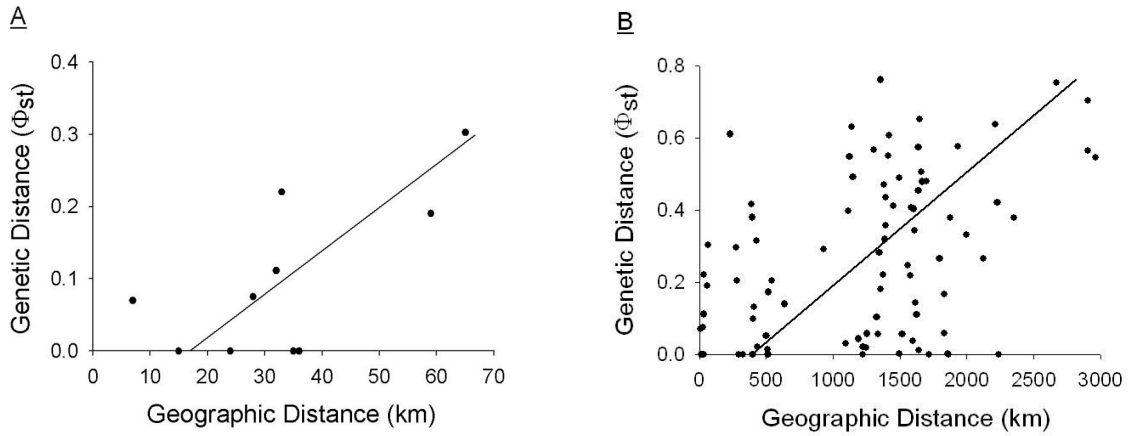


Fig. 9 Isolation-by-distance plots. **A** Spermonde Archipelago; $y=-0.1071+6.106^{-03}x$, $r=0.69$, $R^2=0.48$, $p=0.0427$. **B** Indo-Malay Archipelago; $y=-0.1021+3.016^{-04}x$, $r=0.45$, $R^2=0.2$, $p=0.0104$. Negative genetic distances were set to zero.

Table 6 Hierarchical Analysis of Molecular Variance (AMOVA) groupings. Ad: Adi; BL: Barrang Lompo; Ce: Cebu; Gi: Gilli Trawangan; Ko: Komodo; Kp: Kapoposang; La: Lanyukang; Ma: Manado; PS: Pulau Seribu; Sb: Saboeda; Se: Pulau Sembilan; Sm: Samalona; Sr: Sarappokeke; Ti: Tilamuta

Grouped Populations	Φ_{ct}	***p***
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad) (PS) (Sb) (Ti)	0.31994	0.00712
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad) (PS) (Se) (Sb) (Ti)	0.28380	0.00974
(Ad, BL, Ce, Gi, Ko, Kp, Ma, PS, Sb, Se, Sm, Sr, Ti)	0.25919	0.00000
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb) (PS) (Ti)	0.23216	0.01870
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb) (PS) (Se) (Ti)	0.19836	0.03345
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Se) (PS) (Ti)	0.17017	0.03227
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb, Ti) (PS)	0.15449	0.04465
(BL, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb) (Ce) (PS) (Ti)	0.14260	0.08523
(BL, Gi, Ko, Kp, Se, Sm, Sr) (Ad, Sb) (Ce, Ma) (PS) (Ti)	0.13662	0.06510
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Ti) (Se) (PS)	0.12492	0.07738
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Se, Ti) (PS)	0.11662	0.05212
(BL, Gi, Ko, Kp, PS, Se, Sm, Sr,) (Ad, Sb) (Ce, Ma) (Ti)	0.05764	0.06165

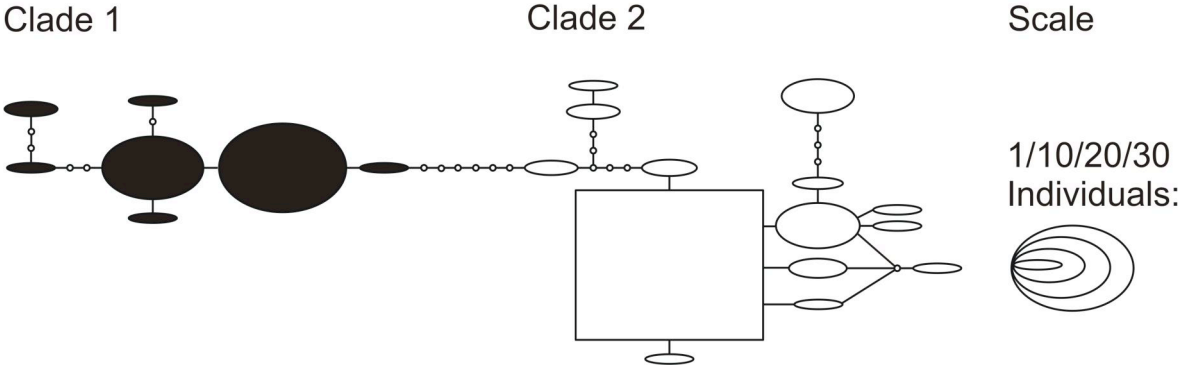


Fig. 10 Unrooted minimum-spanning network showing 21 haplotypes grouped into two clades. Square: ancestral haplotype (n=59). Size of ovals/square: proportional to haplotype frequencies. Lines represent single mutational steps, empty circles missing intermediate haplotypes.

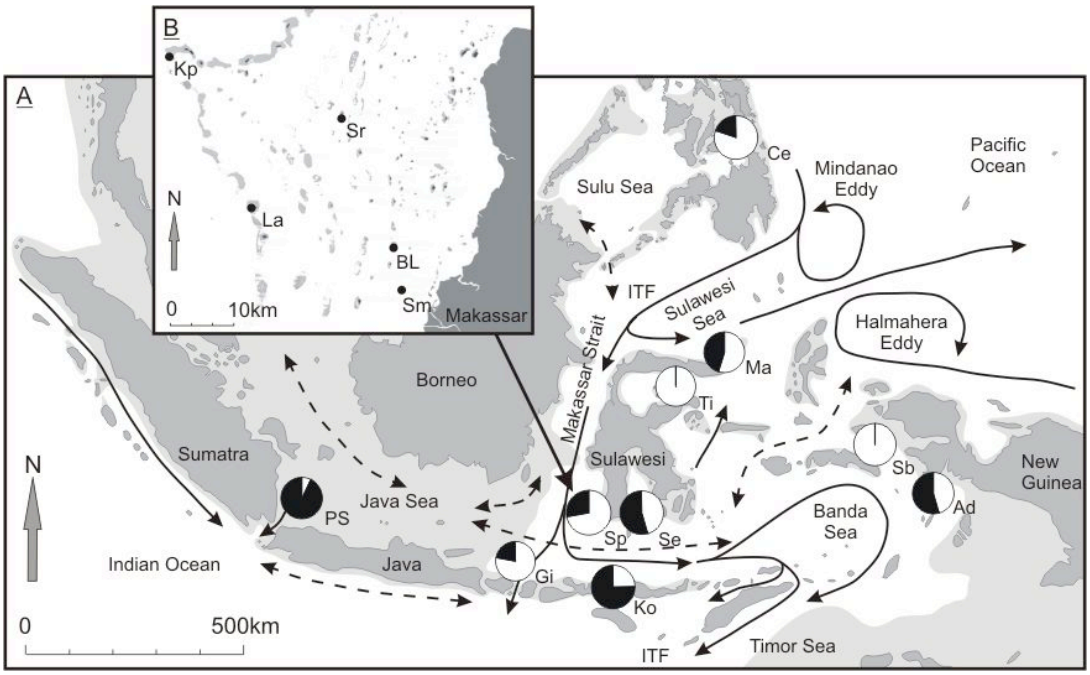


Fig. 11 A Sampling sites in the Indo-Malay Archipelago. Ad: Adi; Ce: Cebu; Gi: Gilli Trawangan; Ko: Komodo; Ma: Manado; PS: Pulau Seribu; Sb: Saboeda; Se: Pulau Sembilan; Sp: Spermonde; Ti: Tilamuta. Pie diagrams show frequencies of clades 1 and 2 (see Fig. 3; Spermonde data pooled). Arrows indicate dominant (solid) and seasonally changing (dashed) currents. Shaded areas represent shelf regions exposed during the Pleistocene (taken from Voris 2000). ITF: Indonesian Throughflow. **B** Sampling sites in the Spermonde Archipelago. BL: Barrang Lompo; Kp: Kapoposang; La: Lanyukang; Sm Samalona; Sr: Sarappokeke. Shaded areas represent present day reefs (taken from Landsat ETM+ satellite image, acquisition year 2002).

4. Discussion

Polyp growth

The majority of coral species are known to grow indeterminately and thus theoretically have unlimited size (Bak and Meesters 1998). Previous studies have shown growth rates to be determinate in the detached life-cycle phase of a number of mushroom coral species: in *Fungia granulosa* (Chadwick-Furman et al. 2000), *Ctenactis echinata*, *Fungia scutaria*, *Fungia fungites* and *Fungia (Danafungia) spp.* (Goffredo and Chadwick-Furman 2003) growth rates decreased as coral size increased.

The Gulland and Holt Plot showed a high variability in the growth rates of the individual *H. actiniformis* polyps investigated in this study, as evidenced by the large degree of scatter around the line of best fit (Fig. 2A). However, a linear relationship between growth rates and polyp length was found, indicating non-seasonal von Bertalanffy type growth. Moreover, the assessment of coral shape parameters revealed that the width:length ratio did not change as individuals aged, growth in mass was thus isometric. Similar growth patterns have been recorded for *F. fungites*, *F. granulosa* and *Danafungia* spp. (Goffredo and Chadwick-Furman 2003). In contrast, the solitary polyps of *F. paumotensis* (Bablet 1985) and *F. scutaria* (Goffredo and Chadwick-Furman 2003) elongate as they grow.

The von Bertalanffy growth function has proven a useful model in the study of a number of invertebrate species with isometric growth where growth declined with size/age (Brey et al.

1990; Plaut and Fishelson 1991; Clasing et al. 1994), and indeed a number of solitary scleractinian coral species (Bablet 1985; Chadwick-Furman et al. 2000; Goffredo and Chadwick-Furman 2003; Goffredo et al. 2004). The asymptotic individual length predicted by the application of a Munro Plot (L_∞ = 163mm) was in fact considerably smaller than the maximum size measured in the field (L = 185mm) and the longest *H. actiniformis* measured by Abe (1940) in Palau (L = 195mm). The size frequency data collected during the course of this study and the work of Abe (1937, 1939, 1940) however confirmed the rarity of such large polyps; we thus deem the model to nevertheless be representative of the overall population. Furthermore, the growth constant calculated in the present study (k = 0.0741) is similar to that estimated by Abe in Palau (k = 0.0839) and within the range of constants available in the published literature for the anthocyathi of other mushroom coral species (reviewed in Chadwick-Furman et al. 2000).

Existing variations in growth rate constants determined for mushroom corals have in the past been attributed to a negative relationship between growth rate and latitude (Goffredo and Chadwick-Furman 2003). The consideration of growth performance indices, i.e. how fast a polyp will attain its eventual body size, however does not seem to support this observation (Table 1). Moreover, in *F. fungites*, the only species for which data from several latitudes is available to date, a high Φ value for Eilat (3.5) contrasts a low Φ value for Sharm el Sheik (3.0), despite the relative similarity in estimated growth constants (0.049 / 0.088 respectively) when compared to the growth constant measured at the Great Barrier Reef (0.215). This is due to the large difference in estimated maximum length at the two sites (250mm / 107mm), an observation which, considering the proximity of the two locations, requires further study. With respect to *H. actiniformis,* the growth parameters estimated in the present study gave a lower Φ value then those calculated from the data available for Palau (3.3 and 3.5 respectively), a pattern which may be due to the fact that growth was studies at 10-12m and 0-2m depth respectively. Indeed, although declining growth rates with depth have been shown for scleractinian coral species in the past (e.g. see Huston 1985; Boscher and Meesters 1992; Miller 1995), this parameter has received little attention in existing studies of mushroom coral population dynamics.

Population size and age structure

A dominance of small colonies and of only few specimens surviving to large sizes has been shown for a number of apparently healthy coral populations (Hughes and Jackson 1985; Lewis 1989; Babcock 1991; Vermeij and Bak 2002; Adjeroud et al. 2007), with authors of studies showing less pronounced left-skewed size-frequency distributions frequently suggesting that high mortalities occurred when coral colonies were too small to be sampled effectively (Grigg 1984; Johnson 1992). Indeed, in previous studies of mushroom corals, a high abundance of attached polyps and subsequent exponential decrease in the frequency of individuals with age was inferred for *Fungia granulosa* (Chadwick-Furman et al. 2000), *Ctenactis echinata*, *Fungia fungites* and *Danafungia* spp. (Goffredo and Chadwick-Furman 2003), despite the fact that only a limited number of attached polyps were actually found and measured. The authors further suggested that a mushroom coral population in steady state with no age cohorts missing or over-represented should show an exponential decline in numbers with age, with the rate of decrease varying among taxa. Focusing our attention on young attached polyps enabled us to confirm that in populations of *H. actiniformis* the age structure is indeed characterised by a marked decrease in frequency of individuals with age, apparently supporting the assumptions of previous authors. However, our results did not show a uniform exponential decline in numbers, but rather indicated a pronounced effect of the different life cycle stages on the overall population structure, with high mortality rates for attached polyps (Z=0.47-0.63) and much lower ones for detached polyps (Z=0.047-0.080).

Whilst there was a lack of statistically significant differences in age-frequency distributions as well as mortality rates between sites, only few polyps survived to an age of 40 years at Samalona and Barrang Lompo, whereas at Bone Baku a noteworthy number of polyps aged 30-40 years was found. This was reflected in the higher mean age and its lower coefficient of variation at Bone Baku when compared to Samalona and Barrang Lompo. Since all sites

were located close to one another and exposed to similar environmental influences, the higher prevalence of coral rubble at Barrang Lompo and Samalona (Fig. 5) is the most likely explanation. The unstable nature of coral rubble as a substrate may primarily impact the survival rates of the largest polyps: Abe (1939) showed that *H. actiniformis* polyps of 6-10cm are able to inflate their tissue and overturn to a normal position when turned upside down. Due to the exponential increase in skeletal weight with polyp length it is probable that the larger polyps are unable to do so, hence making them more vulnerable on an unstable substrate.

Asexual budding has been proposed as a mechanism enabling fungiid populations to recover and recolonise sites following disturbances (Krupp et al. 1992). Indeed, a recent study of *Fungia fungites* populations revealed that the importance of asexual budding from parent polyps vs. budding from sexually recruited reef stalks depended on the level of site disturbance: at the exposed site, a large number of dead parent skeletons with attached buds were found, whereas at the sheltered site only reef stalks were encountered (Gilmour 2004). Lying in the path of the north-west monsoon, the study sites were exposed to the most severe wave action encountered in the Spermonde Archipelago, yet only 2.5% of the attached polyps censured during the transect surveys were found growing on a single live adult polyp at Bone Baku. Boschma (1922) observed similar buds on the undersides of live polyps during an expedition in Java, Indonesia, but concluded them to be an 'abnormal phenomenon'. Moreover, mechanical disturbance as a result of shifting rubble pieces would have been higher at Samalona and Barrang Lompo when compared to Bone Baku, yet no polyps attached to a parent poly or skeleton were recorded at these sites. Moreover, the ability to grow asexual buds on a dead parent skeleton was not observed at any of our sites, potentially rendering *H. actiniformis* less resilient to disturbances compared to other species in the family Fungiidae.

Instead, we found the production of asexual buds via the repeated production of anthocaulus stalks from a single sexual recruit to be of major importance in the population dynamics of *H. actiniformis*. Such prolific budding by reef stalks seems to be an occurrence unique to *H. actiniformis*: the sexual recruits of other mushroom corals only produce a single stalk which may occasionally regenerate tentacles (Chadwick-Furman and Loya 1992; Gilmour 2004). The existence of clustered anthocaulus stalks in *H. actiniformis* was first described by Boschma (1922) and Abe (1937), who termed them anthocormi, but their importance in this species life cycle was hitherto unknown.

However, the high natural mortality rates of the attached polyps we measured in the field limited the contribution of these asexual recruits to the population beyond the initial stage of bud production. Similarly, no detached polyps with a common genotype were found despite prolific asexual budding on dead parent skeletons at a site in the Dampier Archipelago, Australia (Gilmour 2002a). Whilst chronic sedimentation seemed responsible for the high mortality rates of the small polyps in the Dampier Archipelago, competition by other sessile macro-organisms (e.g. Chadwick-Furman and Loya 1992; McCook et al. 2001), as well as predation by corallivorous fish and gastropods (e.g. Bak and Engel 1979) are frequently responsible for the limited survival of young coral recruits. It is likely that sedimentation from the plume of the rivers Jene Berang and Maros as well as competition by increasingly prolific algal growth on the nearshore reefs of the Spermonde Archipelago (J. Jompa, personal communication) and predation all interacted to cause in the high mortality rates we observed during the course of this study. The potentially lower level of physical disturbance at Bone Baku did not significantly reduce the mortality rates of attached polyps, although a higher average number of buds per cluster as well as a larger size at first detachment was noted here. Similarly, sexual recruits detached earlier and were thus of a smaller size at a site exposed to physical disturbances in Australia (Gilmour 2004).

Overall we found similar population size and age structures as well as mortality rates at the three sites investigated, and thus deem our results to be representative of *H. actiniformis* populations at sites exposed to the hydrodynamic regimes of the north-western monsoon in the Spermonde Archipelago. The fact that the differences we did observe could be correlated with differences in rubble cover, and the observation that such effects are likely to vary

according to the life history characteristics of mushroom coral species calls for future investigations focussing specifically on this topic.

Cohort biomass and potential yield

The Beverton and Holt model effectively estimates the point (i.e. age of the corals) at which population biomass is maximized allowing for the highest yield to be obtained through harvest. Its application to coral species has been attempted in a number of studies in both tropical and temperate ecosystems (Table 1), and has been hailed as a solution to the current shortcomings in the management of exploited coral populations (Chadwick-Furman et al. 2000; Goffredo et al. 2004). Whilst it has been successfully used to manage the harvest of the black coral in Hawaii (Grigg 2001), and to advise the management of red coral harvesting in Spain (Tsounis et al. 2007), an application to a species exploited for the live coral aquarium trade has to date not been attempted.

Table 2 Ages at the attainment of maximum biomass/recruit known for anthozoans

Reference	**Species**	**Location**	**Age (yrs)**
Goffredo & Chadwick-Furman 2003	*Fungia scutaria*	Eilat, Egypt	5
Goffredo et al. 2004	*Balanophyllia europea*	Calafurnia, Italy	6
Grigg 1984; Ross 1984	*Pocillopora verrucosa*	Cebu, Philippines	6
Goffredo & Chadwick-Furman 2003	*Fungia fungites*	Eilat, Egypt	8
Chadwick-Furman et al. 2000	*Fungia granulosa*	Eilat, Egypt	9
Goffredo & Chadwick-Furman 2003	*Danafungia spp.*	Eilat, Egypt	10
Goffredo & Chadwick-Furman 2003	*Ctenactis echinata*	Eilat, Egypt	14
Present study	***Heliofungia actiniformis***	**Sulawesi, Indonesia**	**20**
Grigg, 1976	*Anthipathes dichotoma*	Hawaii	28
Grigg, 1976	*Corallium secundum*	Hawaii	34
Tsounis et al. 2007	*Corallium rubrum*	Cap de Creus, Spain	98

According to our estimates, the minimum age for removal of *H. actiniformis* by the aquarium trade in the Spermonde Archipelago should not be less than 20 years, corresponding to a size of about 12-13cm. Due to the low natural mortality rates of detached polyps, *H. actiniformis* reached the point of maximum yield/recruit significantly later than related mushroom coral species (5-14 years, Table 2), thus making it more vulnerable to overexploitation.
Abe (1937) observed spawning in *H. actiniformis* polyps measuring 8cm in length, but not in those measuring 7cm. Taking this estimate as the size at first reproduction, a size limit of 12-13cm would thus also ensure a sufficient buffer period for sexual reproduction and ensure a continuous supply of larvae. This would sustain the population in the long-term, and fulfill one of the major assumptions of the Beverton and Holt model, that harvest does not affect recruitment.
Another assumption underlying the application of this model is that the population investigated is in a steady state, which implies that none of the age classes should be over- or under-represented. As discussed above, we did however note an apparent under-

representation of polyps aged 30-45 years at Barrang Lompo and Samalona. It follows that (1) whilst the present study seems to give an accurate description of present day *H. actiniformis* demographic traits in the Spermonde Archipelago as evidenced by the replicability of our findings, the results obtained through the above application of the Beverton and Holt need to be interpreted with a certain amount of caution and (2) they may only hold as long as reefs are not degraded further.

Small scale genetic differentiation: Spermonde Archipelago

The significant genetic structure within the Spermonde Archipelago points to limited larval exchange on a small scale: the average distance over which limited dispersal was noted in this study is 52 km. Similarly restricted gene flow in brooding coral species has been found in other studies. For instance *Seriatopora hystrix* exhibits high levels of genetic differentiation along a 90 km stretch of the Great Barrier Reef (Ayre and Dufty, 1994) and *Pocillopora damnicornis* shows significant genetic subdivision among seven sites surrounding Lord Howe Island on the East Coast of Australia separated by only 1-6.5 km (Miller and Ayre, 2004). Previous studies on the genetic structure of marine population genetics within Spermonde Archipelago found no restricted gene flow for the giant clam *Tridacna crocea* (Nuryanto and Kochzius 2006) and the stomatopod *Haptosquilla pulchella* (Barber et al. 2002) using mitochondrial COI sequences. However, both species have significantly longer planktonic larval phases: up to 14 days for *T. crocea* (Lucas, 1994) and 4-6 weeks for *H. pulchella* (Barber et al. 2002).
The significantly different pairwise Φ_{st} values for the sites with the largest geographic separation and the fact that the data follow the isolation by distance model indicate that geographic separation is an important factor for the degree of larval connectivity between the populations sampled in Spermonde (Fig. 9, A). However, a closer look at the data also points towards an influence of local current patterns on local larval exchange patterns. Although detailed information on the oceanography of the Spermonde Archipelago is lacking, it is clearly influenced by the ITF, which passes through the Strait of Makassar. Sites located at or near to the shelf edge are likely to be subjected to stronger currents than those in shallower water located near the coastline (for a detailed bathymetric map of the Spermonde Archipelago see de Klerk, 1983). This may explain the lack of genetic structure found between the outershelf and midshelf populations of Kapoposang, Lanyukang, and Sarappokeke, which have a maximal geographic separation of 36 km. In contrast, gene flow between Samalona (nearshore) and Lanyukang (midshelf), separated by 33 km, is limited. Furthermore, whereas a number of private haplotypes were found in the nearshore populations, none were present at the midshelf and outershelf sites. Such private haplotypes are most likely to develop in populations which have a limited exchange of larvae with surrounding populations (Barber et al. 2006), again supporting the notion of more isolated nearshore populations of *H. actiniformis* in the Spermonde Archipelago.

Large scale genetic differentiation: Indo-Malay Archipelago

At the large scale of the Indo-Malay Archipelago, long-distance gene flow between the Central Visayas and populations as far away as those in the Flores Sea was contrasted by regionally isolated populations in New Guinea, Tomini Bay, and the Java Sea. The presence of one large population with only limited genetic structure spanning the central part of the Indonesian Seas further supports the importance of current patterns in *H. actiniformis* larval dispersal: all populations lie very close to or directly in the path of the ITF current. A similar lack of genetic differentiation over a distance of 700 km was found for the coral *Pleisiastrea versipora* within the Ryuku Archipelago by Rodriguez-Lanetty and Hoegh-Guldberg (2002), who suggested dispersal aided by the strong Kuroshio Current as a likely explanation. Furthermore, within the Indo-Malay Archipelago, high levels of gene flow have previously been found along the path of the ITF: from North-Eastern Borneo to Southern Sulawesi for the prawn *Paneus monodon* (Sugama et al. 2002), from Northern Sulawesi and Northern Borneo to South Sulawesi for the boring giant clam *Tridacna crocea* (Nuryanto and Kochzius,

2006), from Northern Sulawesi to the Flores Sea for the stomatopods *Haptosquilla glyptocerus* and *Gonodactylinus viridis* (Barber et al. 2006), and from Cebu in the Philippines to West Timor for the pink anemonefish *Amphiprion perideraion* (Hamid 2007).
Considering the ITF reaches current strengths of up to 1m s^{-1} (Wyrtki 1961) and the potentially long competency periods of brooded coral larvae (Richmond 1989), it may be conceivable that *H. actiniformis* larvae are carried all the way from the Philippines to the distant reefs in the Flores Sea. However, it is more likely that dispersal is occurring via a series of stepping stones (Kimura and Weiss 1964). This notion is given support by the significant isolation by distance analysis at the large scale of the Indo-Malay Archipelago and the genetic break between the Flores Sea and New Guinea. Although the populations at Adi and Saboeda are potentially connected to those in the Flores Sea by an offshoot of the ITF (Fig. 11), potential stepping stones are lacking over considerable distances in this region. The limited number of samples collected in New Guinea combined with the curious presence of genetic differentiation between Adi and Saboeda, despite the islands' proximity and the presence of linking currents between the two sites (Gordon, 2005), does however suggest that the results should be treated with some caution.
H. actiniformis populations in Tomini Bay were genetically dissimilar to all other populations sampled in this study. This is a region with numerous endemic species (Wallace et al. 2003), and a similar pattern of restricted gene flow with surrounding populations was found in *H. pulchell*a by Barber et al. (2002). A likely explanation lies in the oceanographic properties of the area: there are no major currents driving an exchange of water masses with the neighbouring Malukku Sea (Gordon 2005). However, a genetic break between the neighbouring Java and Flores Sea *H. actiniformis* populations was also found, despite the presence of seasonally reversing currents in this region (Fig. 11). Indeed, a number of studies found similar genetic structuring between populations lying on the Sunda Shelf's Java Sea and those in the Flores Sea (Lourie et al. 2005; Barber et al. 2006; Nuryanto & Kochzius 2006; Timm & Kochzius, 2006; Hamid 2007). The most likely explanation is that these populations may still carry the genetic signature of re-colonisation events on the Sunda Shelf at the end of the last glacial period. As *H. actiniformis* is absent from the South China Sea but present on the western shores of Sumatra and Java (Veron 2000), re-colonisation may have come from populations in the Indian Ocean. This would agree with results from Timm and Kochzius (2006), who found high levels of gene flow between *Amphiprion ocellaris* populations on the Sunda Shelf and in the Indian Ocean. However, an extension of the current sampling regime into the Indian Ocean and at more sites on the Sunda Shelf would be needed to confirm this hypothesis.
The revealed pattern of two major clades (Fig. 10) nevertheless further supports this hypothesis. On the Sunda Shelf 93% of the haplotypes sampled belonged to clade 2. Also striking are the populations in Tomini Bay and at Saboeda (New Guinea), both of which only contained clade 1 haplotypes. Molecular genetic analyses of starfish (Williams and Benzie 1998; Benzie 1999; Kochzius et al. 2006), crustaceans (Duda and Palumbi 1999; Barber et al. 2002; Benzie et al. 2002) and fish (Bernardi et al. 2001, Timm & Kochzius 2006) all found a clear break between Indian and Pacific Ocean populations. In *H. actiniformis*, clade 1 haplotypes may have originated in the Pacific Ocean and the clade 2 haplotypes in the Indian Ocean. Subsequent dispersal and mixing processes could have generated the modern day distribution patterns of the two clades. This would also agree with existing taxonomic appraisals of scleractinian corals in the region, which have indicated biogeographic patterns consistent with speciation events in the Indian and Pacific Oceans and ensuing dispersal into the central Indo-West Pacific (Wallace, 1997).
Furthermore, in terms of haplotype diversity, more diverse populations were found in these areas of presumed mixing, with Lanyukang (Spermonde) > Barrang Lompo (Spermonde) > Komodo, than on the periphery, where Saboeda < Adi < Pulau Seribu (Table 2). Patterns of nucleotide diversity somewhat mirrored these findings, with high values for Komodo > Manado > Sarapokeke (Samalona) / Pulau Sembilan where a frequent input of larvae from neighbouring populations is more likely than at the sites with lower values: Tilamuta < Pulau Seribu < Saboeda (Table 3). Benzie et al. (2002) likewise found diversity measures to be five times that of the average in the geographic centre of *Penaeus mondon* distribution and

suggested the evolution of genetic variants in peripheral populations and their subsequent migration into the Indo-Malay Archipelago to be responsible for this pattern. Conspicuous in the present study is the population at Adi off western New Guinea. Here one of the lowest haplotype diversities (0.5) was contrasted by the highest nucleotide diversity (0.014) we found for all *H. actiniformis* populations investigated. It is feasible that haplotype variation is so limited due to the extreme rarity of mixing events at this location, but that the occasional input of larvae comes from three very different sources: the nearby Malukku Sea, the Banda Sea (via the ITF) and the Torres Strait. However, more detailed information on local oceanographic patterns and more samples from New Guinea are needed to draw firm conclusions.

Conclusions and management implications

The emerging pattern of *H. actiniformis* genetic population structure is thus highly complex, with the underlying pattern of isolation by distance as expected confounded by historical and contemporary oceanographic factors, especially at the scale of the Indo-Malay Archipelago (Fig. 9, B). The results do however for the first time give an indication of the present-day levels of population connectivity for *H. actiniformis* and presumably scleractinian coral species with similar ecological features in the Indo-Malay Archipelago, and can thus help to inform conservation and trade management decisions.

The apparent larval seeding of downstream ITF populations has several management implications. Firstly, harvesting at upstream populations should be limited and done with caution: over-harvested populations are likely to have slower recovery rates due to the more limited input of larvae into the system. Secondly, no-take areas at these locations are particularly important as they may serve as larval source regions. Although further research considering scleractinian coral species with a variety of reproductive modes is needed, the results of this study suggest existing reserves such as the chain of marine protected areas along the eastern and southern coast of Negros (Philippines), the Turtle Islands Park in northeast Borneo (Malaysia) and the National Park in Bunaken (Indonesia) (Spalding et al. 2002) should be strengthened, enlarged and ideally multiplied to support conservation efforts in the coral triangle.

Whilst it might be tempting to set less stringent quotas for those populations apparently lying downstream of potential source populations, restricted gene flow may occur within local reef systems, as evidenced by the pattern found within the Spermonde Archipelago in the present study, and also for coral populations within the Great Barrier Reef (Ayre et al. 1997, 2000). In the case of the Spermonde Archipelago, more stringent quotas should be set for nearshore reefs, where a larval input via the ITF is less likely than for mid- and outershelf reefs. More generally, the results underline the importance of considering the detailed characteristics of each harvesting area separately to achieve long term sustainability.

Finally, as for the upstream populations, isolated populations not connected by suitable stepping stones and/or sufficiently strong current regimes, should be treated with extreme prudence when harvesting quotas are set. Indeed, the government of Gorontalo province is currently considering whether to encourage fishermen to engage in the ornamental trade (personal communications). Since the population in Tomini Bay is genetically isolated, collection of *H. actiniformis* and presumably a number of scleractinian coral species should be restricted and carefully monitored.

5. References

5.1 Population Dynamics

Abe, N., 1937. Post-larval development of the coral *Fungia actiniformis* var. palawensis Doederlein. Palau Trop. Biol. Station Stud. 1, 73-93.

Abe, N., 1939. Migration and righting reaction of the coral, *Fungia actiniformis* var. palawensis Doederlein. Palau Trop. Biol. Station Stud. 4, 671-694.

Abe, N., 1940. Growth of *Fungia actiniformis* var. palawensis Doederlein and its environmental conditions. Palau Trop. Biol. Station Stud. 5, 105–145.

Adjeroud, M., Pratchett, M.S., Kospartov, M.C., Lejeusne, C., Penin, L., 2007. Small-scale variability in the size structure of scleractinian corals around Moorea, French Polynesia: patterns across depths and locations. Hydrobiologia 589, 117-126.

Bablet, J.P., 1985. Report on the growth of a scleractinia (*Fungia paumotensis*). In: Proceedings of the 5th International Coral Reef Symposium, Tahiti, Vol. 4, 361-365.

Bellwood, D.R., Hughes T.P., Folke C., Nystroem M., 2004. Confronting the coral reef crisis. Nature 429, 827-833.

Babcock, R.C., 1991. Comparative demography of three species of scleractinian corals using age- and size-dependent classifications. Ecol. Monogr. 61, 225–244.

Bak, R.P.M., Engel, M.S., 1979. Distribution, abundance and survival of juvenile hermatypic corals (Scleractinia) and the importance of life history strategies in the parent coral community. Mar. Biol. 54, 341-352.

Bak, R.P.M., Meesters, E.H., 1998. Coral population structure: the hidden information of colony size frequency distributions. Mar. Ecol. Prog. Ser. 162, 301–306.

Baquero, J., 1991. Scleractinians or stony corals and the aquarium trade. Part I. Sea Wind, 5, 6-12.

Beverton, R.J.H., Holt, S.V., 1957. On the dynamics of exploited fish populations. Fish. Invest. Minist. Agric. Fish. Food Ser. II 19, 1-553.

Boschma, H., 1922. On budding and coalescence of buds in *Fungia fungites* and *Fungia actiniformis*. Proc. Kon. Ned. Akad. Wet. Amsterdam 24, 257-268.

Bosscher, H., Meesters, E.H., 1992. Depth related changes in the growth rate of *Montastrea annularis*. In: Proceedings of the 7th International Coral Reef Symposium, Guam, Vol. 1, 507-512.

Brey T., Arntz W.E., Pauly D., Rumohr H., 1990. *Arctica* (*Cyprina*) *islandica* in Kiel Bay (Western Baltic): growth, production and ecological significance. J. Exp. Mar. Biol. Ecol. 136, 217-235.

Chadwick-Furman, N.E., Loya, Y., 1992. Migration, habitat use, and competition among mobile corals (Scleractinia: Fungiidae) in the Gulf of Eilat. Red Sea Mar. Biol. 114, 617–623.

Chadwick-Furman, N.E., Goffredo, S., Loya, Y., 2000. Growth and population dynamic model of the reef coral *Fungia granulosa* Klunzinger, 1879 at Eilat, northern Red Sea. J. Exp. Mar. Biol. Ecol. 254, 199-218.

Clasing, E., Brey, T., Stead, R., Navarro, J., Asencio, G., 1994. Population dynamics of *Venus antiqua* (Bivalvia: Veneracea) in the Bahia de Yaldad, Isla de Chiloe, Southern Chile. J. Exp. Mar. Biol. Ecol. 177, 171–186.

Connell, J.H., Hughes, T.P., Wallace, C.C., 1997. A 30-year study of coral abundance, recruitment, and disturbance at several scales in space and time. Ecol. Monogr. 67, 461–488.

de Klerk, L.G ., 1983. Sea levels, reefs and coastal plains of Southwest Sulawesi, Indonesia; a morphogenetic-pedological study. Dissertation, State University of Utrecht, 172 pp.

Edmunds, P.J., Elahi, R., 2007. The demographics of a 15-year decline in cover of the Caribbean reef coral *Montastrea annularis*. Ecol. Monogr. 77, 3-18.

Edinger, E.N., Jompa, J., Limmon, G.V., Widjatmoko, W., Risk, M.H., 1998. Reef degradation and coral biodiversity in Indonesia: effects of land-based pollution, destructive fishing practices and changes over time. Mar. Pollut. Bull. 36, 671-630.

Erdmann, N., 1995. The ABC guide to coral reef fisheries in Southwest Indonesia, Sulawesi. In: NAGA, the ICLARM Quaterly, 129 pp.

Fong, P., Glynn, P.W., 1998. A dynamic size-structured population model: does disturbance control size structure of a population of the massive coral *Gardineroseris planulata* in the Eastern Pacific? Mar. Biol. 130, 663-674.

Fox, H.E., 2004. Coral recruitment in blasted and unblasted sites in Indonesia: assessing rehabilitation potential. Mar. Ecol. Prog. Ser. 269, 131-139.

Gardener, T.A., Cote I., Gill J.A., Grant A., Watkinson A.R., 2003. Long-term region-wide declines in Caribbean corals. Science 301, 958-960.

Gilmour, J.P., 2002a. Substantial asexual recruitment of mushroom corals contributes little to population genetics of adults in conditions of chronic sedimentation. Mar. Ecol. Prog. Ser. 235, 81-91.
Gilmour, J.P., 2002b. Acute sedimentation causes size-specific mortality and asexual budding in the mushroom coral, *Fungia fungites*. Mar. Freshwater Res. 53, 805-812.
Gilmour, J.P., 2004. Size-structures of populations of the mushroom coral *Fungia fungites*: the role of disturbance. Coral Reefs 23, 494-504.
Glassom, D., Zahai, D., Chadwick-Furman N.E., 2004. Coral recruitment: a spatio-temporal analysis along the coastline of Eilat, northern Red Sea. Mar. Biol. 144, 641-651.
Gleason, M.G., 1996. Coral recruitment in Moorea, French Polynesia: the importance of patch type and temporal variation. J. Exp. Mar. Biol. Ecol. 207, 79-101.
Goffredo, S., Chadwick-Furman, N.E., 2003. Comparative demography of mushroom corals (Scleractinia: Fungiidae) at Eilat, northern Red Sea. Mar. Biol. 142, 411-418.
Goffredo, S., Mattioli G., Zaccanti F., 2004. Growth and population dynamics model of the Mediterranean solitary coral *Balanophyllia europaea* (Scleractinia, Dendrophylliidae). Coral Reefs 23, 433-443.
Green, E.P, Shirley, F., 1999. The global trade in corals. World Conservation Monitoring Centre. World Conservation Press, Cambridge UK, 60 pp.
Grigg, RW., 1976. Fisheries management of precious and stony corals in Hawaii. UNIHI Sea Grant Tr 77/03, 1-48.
Grigg, R.W., 1984. Resource management of precious corals: a review and application to shallow water reef building corals. P.S.Z.N.I. Mar. Ecol. 5, 57–74.
Grigg, R.W., 2001. Black coral: history of a sustainable fishery in Hawaii. Pac. Sci. 55, 291-299.
Harriott, V.J., 1992, Recruitment patterns of scleractinian corals in an isolated sub-tropical reef system. Coral Reefs 11, 215-219.
Hoeksema, B.W., 1989. Taxonomy, phylogeny and biogeography of mushroom corals (Scleractinia: Fungiidae). Zool. Verh. Leiden 254, 1–295.
Hoeksema, B.W., Moka, W., 1989. Species assemblages and phenotypes of mushroom corals (Fungiidae) related to coral reef habitats in the Flores Sea. Net. J. Sea Res. 23, 149-160.
Hoeksema, B.W., 2004. Impact of budding on free-living corals at East Kalimantan, Indonesia. Coral Reefs 23, 492.
Hughes, T.P., 1984. Population dynamics based on individual size rather then age: a general model with a reef coral example. Am. Nat. 123, 778–795.
Hughes, T.P., Jackson, J.B.C., 1985. Population dynamics and life histories of foliaceous corals. Ecol. Monogr. 55, 141–166.
Hughes, T.P., Connell, J.H., 1987. Population dynamics based on size or age? A reef-coral analysis. Am. Nat. 129, 818-829.
Hughes, T.P., 1989. Community structure and diversity of coral reefs: the role of history. Ecology 70, 275-279.
Hughes, T.P., Bellwood, D.R., Connolly, S.R., 2002. Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. Ecol. Lett. 5, 773-784.
Huston, M., 1985. Variation in coral growth rates with depth at Discovery Bay, Jamaica. Coral Reefs 4, 19-25.
Johnson, K.G., 1992. Population dynamics of a free-living coral: recruitment, growth and survivorship of *Manicina areolata* (Linnaeus) on the Caribbean coast of Panama. J. Exp. Mar. Biol. Ecol. 164, 171–191.
Jokiel, P.L., Maragos, J.E., Franzisket, L., 1978. Coral growth: buoyant weight technique. In: Stoddart, D.R., Johannes, R.E. (eds.), Coral reefs: research methods. UNESCO, Paris, p. 529–541.
Knittweis, L., Kraemer, W.E., Timm, J., Kochzius, M., 2007. Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts. Cons. Genetics (submitted).

Knittweis, L., Wolff, M., 2008. Live coral trade impacts on population structure of the mushroom coral *Heliofungia actiniformis* in South Sulawesi, Indonesia: harvest management implications.

Krupp, D.A., Jokiel P.L., Chartrand T.S., 1992. Asexual reproduction by the solitary scleractinian coral *Fungia scutaria* on dead parent coralla in Kanehoe Bay, Ohahu, Hawaiian Islands. Ind: Proc. 7th Int. Symp. Coral Reefs 1, 527-534.

Lamberts, A.E., 1978. Coral growth: alizarin method. In: Stoddart, D.R., Johannes, R.E. (eds.), Coral reefs: research methods. UNESCO, Paris, p. 523–527.

Lewis, J.B., 1989. Spherical growth in the Caribbean coral *Siderastrea radians* (Pallas) and its survival in disturbed habitats. Coral Reefs 7, 161–167.

Lewis, H.B., 1997. Abundance, distribution and partial mortality of the massive coral *Siderastrea sidereal* on degrading coral reefs at Barbados, West Indies. Mar. Polut. Bull. 34, 622-627.

McCook, L.J., Jompa, J., Diaz-Pulido G., 2001. Competition between corals and algae on coral reefs: a review of evidence and mechanisms. Coral Reefs 19, 400-417.

Meesters, E.H., Hilterman M., Kardinaal, E., Keetman, M., de Vries M., Bak, R.P.M., 2001. Colony size-frequency distributions of scleractinian coral populations: spatial and interspecific variation. Mar. Ecol. Prog. Ser. 209, 43-54.

Miller, M.W., 1995. Growth of a temperate coral: effects of temperature, light, depth and heterotrophy. Mar. Ecol. Prog. Ser. 122, 217-225.

Moll, H., 1983. Zonation and diversity of Scleractinia on reefs off S.W. Sulawesi, Indonesia. Univ. Leiden Thesis, Leiden, 107 pp.

Munro, H.L., 1982. Estimation of the parameters of the von Bertalanffy growth equation from recapture data at variable time intervals. J. Cons. CIEM 40, 199-200.

Nacua S.S., Alino, P.M., 1994. Comparison on the colonization and recruitment of algae and sessile (sic) on three different types of recruitment plates at two sites in Bolinao, Pangasinan, Philippines. Bull. Mar. Sci. 55, 1347-1348.

Pet-Soede, C., Van Desen W.L.T., Hiddink J.G., Kuyl S., Machiels M.A.M., 2001. Can fishermen allocate their fishing effort in space and time on the basis of their catch rates? An example from Spermonde Archipelago, S.W. Sulawesi, Indonesia. Fisheries Manag. Ecol. 8, 15-36.

Pauly, D., 1984. Fish population dynamics in tropical waters: a manual for use with programmable calculators. ICLARM Stud. Rev. 8. 325pp.

Plaut, I., Fishelson L., 1991. Population structure and growth in captivity of the spiny lobster *Panulirus penicillatus* from Dahab, Gulf of Aqaba, Red Sea. Mar. Biol. 111, 467-472.

Raymakers, C., 2001. Review of trade in live corals from Indonesia. Traffic Europe, Brussels, 18 pp.

Reyes, M.Z., Yap, H.T., 2001. Effect of artificial substratum material and resident adults on coral settlement patterns at Danjugan Island, Philippines. Bull. Mar. Sci. 69, 559-566.

Ross, M.A., 1984. A quantitative study of the stony fishery in Cebu, Philippines. Mar. Ecol. Pubbl. Stn. Zool. Napoli 5, 75-91.

Sparre, P., Venema, S.C., 1998. Introduction to tropical fish stock assessment. FAO Fisheries Technical Paper 306/1. 407pp.

Tomascik, T., Mah, A.J., Nontji, A., Moosa, M.K., 1997. The Ecology of the Indonesian Seas Part One. The Ecology of Indonesia Series Volume VII. Periplus, Singapore, 642 pp.

Tsounis, G., Rossi, S., Gili, J.M., Arntz, W., 2006. Population structure of an exploited benthic cnidarian: the case study of red coral (*Corallium rubrum* L.). Mar. Biol. 149, 1059-1070.

Tsounis, G., Rossi, S., Gili, J.M., Arntz, W., 2007. Red coral fishery at the Costa Brava (NW Mediterranean): case study of an overharvested precious coral. Ecosystems, online first.

von Bertalanffy, L., 1938. A quantitative theory of organic growth (Inquiries on growth laws. II). Hum. Biol.10, 181–213.

Vermij, M.J.A., Bak, R.P.M., 2002. Inferring demographic processes from population size structure in corals. In: Proceedings of the 9th International Coral Reef Symposium, Vol. 1, 598-593.

Wabnitz, C., Taylor, M., Green E., Razak, T., 2003. From ocean to aquarium. UNEP-WCMC, Cambridge, UK, 66 pp.
Wilkinson, C.R., 2004. Status of coral reefs of the world. Global Coral Reef Monitoring Network, Australian Institute of Marine Science, Australia, 316pp.
Yamashiro, H., Nishihira, M., 1998. Experimental study of growth and asexual reproduction in *Diaseris distorta* (Michelin, 1843), a free-living fungiid coral. J. Exp. Mar. Biol. Ecol. 225, 253-267.

5.2 Genetics

Abe N (1937) Post-larval development of *Fungia actiniformis*. Palao Trop Biol Stn Stud 1:73-9
Abe N (1940) Growth of *Fungia actiniformis* var. palawensis Doederlein and its environmental conditions. Palao Trop Biol Stn Stud 2:105–145
Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48(4): 1183-1201
Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillipora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175-187
Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605
Barber PH, Palumbi SR, Erdmann MV, Kasim Moosa M (2002) Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol Ecol 11:659-674
Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution 60(9):1825-1839
Benzie JAH (1999) Genetic structure of coral reef organisms: ghosts of dispersal past. Am Zool 39:131-145
Benzie JAH, Ballment E, Forbes AT, Demetriades NT, Sugama K, Haryanti, Moria S (2002) Mitochondrial DNA variation in the Indo-Pacific populations of the giant tiger prawn *Panaeus monodon*. Mol Ecol 11:2553-2569
Bernardi G, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three spot dascyllus, *Dascyllus trimaculatus*. Mar Biol 138: 457-465
Clement M, Posada D, Crandall KA (2000) TCS: a programme to estimate gene genealogies. Mol Ecol 9:1657-1660
De Klerk LG (1983) Sea levels, reefs and coastal plains of Southwest Sulawesi, Indonesia; a morphogenetic-pedological study. Dissertation, State University of Utrecht
Duda TF Jr, Palumbi SR (1999) Population structure of the black tiger prawn *Panaeus monodon*, among western Indian Ocean and western Pacific populations. Mar Biol 134:705-710
Excoffier L, Laval G, Schneider S (2005) Arlequin version 3.0: an integrated software package for population genetics data analysis. Bioinformatics Online 1:47-50
Forsman ZH, Guzman HM, Chen CA, Fox GE, Wellington GM (2005) An ITS region of Sidastrea (Cnidaria: Anthozoa): is *S. glynni* endangered or introduced? Coral Reefs 24(2):342-347
Gordon AL, Fine RA (1996) Pathways of water between the Pacific and Indian Oceans in the Indonesian seas. Nature 379:146-149
Gordon AL (2005) Oceanography of the Indonesian Seas and their throughflow. Oceanogr 18(4):15-27
Green EP, Shirley F (1999) The global trade in corals. World Conservation Monitoring Centre, World Conservation Press, Cambridge, UK
Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98

Hamid LMO (2007) Genetic population structure of the pink anemonefish *Amphiprion perideraion* (Pomacentridae) in the Indo-Malay Archipelago. MSc Dissertation, University of Bremen

Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the World, vol. 25. Coral reefs Elsevier Science Publishers, Amsterdam, pp133-207

Haq BU, Hardenbol J, Vail PR (1987) Chronology of fluctuating sea levels since the Triassic. Science 235:1156-1167

Hughes TP, Bellwood DR, Connnolly SR (2002) Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. Ecol Lett 5:773-784

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BioMed Cent Genet 6:13

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561-576

Kochzius M, Hauschild J, Kirchhoff S, Meyer-Wachsmuth I, Seidel C (2006) Congruence of genetic population structure in the blue starfish *Linckia laevigata* and its parasite *Thyca crystallina* in the central Indo-Malay Archipelago? In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany,19-22

Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: Hippocampus). Mol Ecol 14:1073-1094

Lucas JS (1994) The biology, exploitation and mariculture of giant clams (Tridacnidae). Rev Fish Sci 2:181-223

Magalon H, Adjeroud M, Veuille M (2005) Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. Mol Ecol 14:1861-1868

Manly BJF (1994) Multivariate statistical methods: a primer. Chapman and Hall, New York

Miller KJ, Ayre DJ (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity 92:557-568

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000) Phylogeographic structure of the false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda Shelf. Mar Biol 137:727-736

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Nishikawa A, Katoh M, Kazuhiko S (2003) Larval settlement and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87-97

Nuryanto A, Kochzius M (2006) Limited gene flow in the giant clam *Tridacna crocea* across the Indo-Malay Archipelago. In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany, 19-22 September 2006

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818

Richmond RH (1989) Competency and dispersal of spawned versus brooded coral planula larvae. Paper presented at the 6th International Coral Reef Symposium, Townsville, Australia, 08-12 September 1988

Rodriguez-Lanetty M, Hoegh-Guldberg O (2002) The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Pleisiastrea versipora* in the Western Pacific. Mol Ecol 11:1177-1189

Spalding MD, Ravilious C, Green EP (2001) World atlas of coral reefs. UNEP World Conservation Monitoring Centre. University of California Press, Berkeley, USA

Sugama K, Haryanti, Benzie JAH, Ballment E (2002) Genetic variation and population structure of the giant tiger prawn, *Penaeus monodon*, in Indonesia. Aquaculture 205:37-48

Takabayashi M, Carter D, Ward S, Hoegh-Guldberg O (1998) Inter- and intra-specific variability in ribosomal DNA sequence in the internal transcribed spacer region of corals. In: Greenwood JG, Hall NJ (eds) Proceedings of the Australian Coral Reef Society 75th Anniversary Conference, The University of Queensland, Brisbane, 1997

Thompson JG, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Timm J, Kochzius M (2006) Strong genetic structure and limited gene flow between populations of the False Clown Anemonefish (*Amphiprion ocellaris*) in the Indo-Malay Archipelago. In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany, 19-22 September 2006

Tomascik T, Mah AJ, Nontji A, Moosa MK (1997) The Ecology of the Indonesian Seas Part One. The Ecology of Indonesia Series Volume VII. Periplus, Singapore

Veron JEN (2000) Corals of the world. AIMS Townsville, Australia

Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral *Acropora cervicornis*: Implications for the recovery of endangered reefs. J Hered 98(1):40-50

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems, time durations. J Biogeogr 27:1153-1167

Wabnitz C, Taylor M, Green E and Razak T (2003) From ocean to aquarium. UNEP-WCMC, Cambridge, UK

Wallace CC (1997b) The Indo-Pacific centre of coral diversity re-examined at species level. Proceedings of the 8th International Coral Reef Symposium, Panama, 24-29 June, 1997

Wallace CC, Paulay G, Hoeksema BW, Bellwood DR, Hutchings PA, Barber PH, Erdmann M, Wolstenholme J (2003) Nature and origins of unique high diversity reef faunas in the Bay of Tomini, Central Sulawesi: The ultimate 'centre of diversity'? Proceedings of the 9th International Coral Reef Symposium, Bali, 23-29 October 2000

White TJ, Bruns T, Lee S, Taylor WJ (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications, Academic Press, San Diego, pp315-322

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 10:506-513

Williams ST, Benzie JAH (1996) Genetic uniformity of widely separated populations on the coral reef starfish *Tinckia laevigata* from the East Indian and West Pacific Oceans, revealed by allozyme electrophoresis. Mar Biol 126:99-107

Williams ST, Benzie JAH (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by colour morphs, mtDNA, and allozyme data. Evolution 52(1):87-99

Wilson JR, Harrison PL (1998) Settlement-competency periods of larvae of three species of scleractinian corals. Mar Biol 131:339-345

Wyrtki K (1961) Physical oceanography of the Southeast Asian waters. Naga Report 2, Scripps Institution of Oceanography, p 195

Appendix 1: Posters and Presentations of Results at Conferences in 2006 / 2007

- Knittweis L., Wolff M. (2007). The need for coral husbandry: a case study of *H. actiniformis* exploitation for the live coral aquarium trade in the Spermonde Archipelago, South Sulawesi, Indonesia.

  1st International Symposium of Coral Husbandry in Public Aquaria, Arnhem, Holland, April 2007.

- Knittweis L. (2006). Participatory management approaches to the aquarium trade in Spermonde Archipelago, South Sulawesi.

  Science Meets Policy for Coastal Management and Capacity Building, Bali, November 2006.

- Ferse S., Kunzmann A., Knittweis L. (2006). Coral reefs are more than fishes, towards the sustainable use of non-fish resources.

  Science Meets Policy for Coastal Management and Capacity Building, Bali, November 2006.

- Knittweis L., Borell E., Jalil H., Jompa J., Richter C. (2006). Land-ocean interaction in coral reefs: stability of photosymbionts along a cross-shelf transect in Spermonde, Indonesia.

  Science Meets Policy for Coastal Management and Capacity Building, Bali, November 2006.

- Knittweis L. (2006). Impacts of the live coral aquarium trade on *H. actiniformis* population dynamics in South Sulawesi, Indonesia.

  International Society for Reef Studies European Meeting, Bremen, Germany, September 2006.

Appendix 2: BSc Thesis submitted by Ms. Sri Asmi Romawanti at Universitas Hasanuddin

UNHAS

SPICE

Reproductive Timing in the Mushroom Coral *Heliofungia actiniformis* at Barrang Lompo Island, Makassar

BSc Student: Sri Asmi Romawanti

Student Number: H411 02 014

Principal Supervisor: Drs. Willem Moka, MSc

Supervisor I: Dr. Chair Rani

Supervisor II: Drs. Munif Hassan, MSc

External Supervisor: Leyla Knittweis

BAB I

PENDAHULUAN

I.1 LATAR BELAKANG

Terumbu karang merupakan habitat kehidupan biota laut yang kaya dengan keanekaragaman hayati. Terumbu karang merupakan salah satu ekosistem tertua di dunia dan diperkirakan ada kira-kira 400 juta tahun yang lalu (Turgeon dan Asch, 2006).

Ekosistem karang memiliki produktivitas dan keanekaragaman jenis hayati yang tinggi. Karena fungsinya sebagai tempat hidup, mencari makan (*feeding ground*), daerah asuhan (*nursery ground*) dan tempat memijah (*spawning ground*) untuk berbagai biota laut (Nontji, 1988).

Salah satu komponen penyusun terumbu karang adalah karang batu (*hard coral*). Karang terdiri atas polip-polip karang yang beraneka warna sehingga memberikan keindahan tersendiri, oleh karena itu beberapa spesies karang dijadikan karang hias untuk akuarium laut (Wabnitz *et al.,* 2003).

Perdagangan karang hias internasional bernilai sekitar 200-330 juta $/tahun (Wabnitz *et al.*, 2003). Sampai awal tahun 1990-an, perdagangan karang hidup meningkat sepuluh kali lipat dan sebagian besar dari karang tersebut masih dikumpulkan dari alam (Green dan Sherly, 1999). Khususnya untuk karang jamur (*Fungiidae*), data pada tahun 1985–1999 menunjukkan bahwa sekitar 25% dari seluruh karang di Indonesia yang diperdagangkan dibawah CITES (*Convention on Trade in Endangered Species*) berasal dari famili *Fungiidae.* Dari famili tersebut *Heliofungia actiniformis* menempati posisi lima besar dari semua spesies karang yang dieksploitasi di pasar (Green dan Sherly, 1999).

Perdagangan karang hias yang semakin meningkat dari tahun ke tahun akan memicu ekstraksi sumber daya karang secara besar-besaran. Dengan melihat fenomena tersebut maka perlu dilakukan upaya pengembangan ke arah budidaya.

Pengembangan budidaya ini diarahkan untuk penyediaan bibit untuk keperluan perdagangan karang. Untuk itu diperlukan informasi tentang berbagai aspek reproduksi karang khususnya karang yanng diperdagangkan di Indonesia.

Pengkajian biologi reproduksi karang secara detail masih sangat terbatas. Ketersediaan informasi tentang aspek reproduksi karang khususnya karang *H. actiniformis* di perairan Spermonde (Pulau Barrang Lompo) sangat diperlukan. Olehnya itu, penelitian

aspek biologi reproduksi karang ini diharapkan dapat dijadikan sebagai dasar untuk pengembangan pembenihan karang hias di masa yang akan datang.

1.2 Tujuan Penelitian

Penelitian ini bertujuan untuk:

1. Mengetahui tipe seksualitas dan mode reproduksi karang *H. actiniformis.*
2. Mengetahui tahapan perkembangan gonad karang *H. actiniformis.*
3. Mengetahui keterkaitan antara fase bulan Hijriah dengan tingkat perkembangan gonad.

1.3 Waktu dan Tempat

Penelitian ini dilaksanakan pada bulan Juli – September 2006, pengambilan sampel karang dilakukan di Perairan Terumbu Karang Pulau BarrangLompo sedangkan analisis histologi dilakukan di Laboratorium Ekotoksikologi dan Fisiologi Biota Laut, Jurusan Ilmu Kelautan, Fakultas Ilmu Kelautan dan Perikanan, Universitas Hasanuddin, Makassar.

1.4 Manfaat Penelitian

Hasil penelitian ini diharapkan sebagai bahan informasi untuk penelitian selanjutnya khususnya yang berkaitan dengan aspek reproduksi karang jamur (*H. actiniformis*).

◄

BAB II

TINJAUAN PUSTAKA

II.1 Uraian Umum

II.1.1 Sistematika dan Morfologi *Heliofungia actiniformis*

Phylum *Coelenterata* memiliki jaringan yang lembut dan sederhana dimana pada bagian atasnya terdapat mulut yang memiliki multifungsi yaitu sebagai tempat pengeluaran (anus) dan memasukkan makanan. Pada rongga gastrovaskuler terdapat mesentri yang mengandung gonad sebagai sel seksual. Mulut dikelilingi tentakel yang berfungsi sebagai pertahanan diri dan menangkap makanan. Tentakel ini mengandung *nematocysts* yang berfungsi melumpuhkan mangsa (Anonim, 2006).

Ordo *Scleractinian* merupakan binatang laut berpolyp yang menghasilkan suatu rangka batu. Rangkanya disusun atas kalsium karbonat yang bersimbiosis dengan alga dan beberapa organisme yang lain (Goreau,1981). Struktur karang batu raksasa (*masive*) dibentuk dari $CaCO_3$. Walaupun semua karang menghasilkan $CaCO_3$, tetapi tidak semua membentuk bangunan batu karang. Karang *Fungia* sp., merupakan karang dengan polyp tunggal yang dapat tumbuh sampai diameter 25 cm (Barnes, 1987; Sumich, 1996).

H. actiniformis memiliki ukuran diameter sampai 50 cm, datar septa besar dengan gigi berbentuk lobata, mulut ditengah dapat mencapai ukuran sampai 3 cm, mempunyai tentakel yang panjang dengan bagian seperti korek api berwarna putih. Hidup tersebar dari *reef flat* hingga kedalaman 20 m dan bersifat soliter (Suharsono, 1996).

Taksonomi *H. actiniformis* menurut (Veron., 1986) yaitu:

Kingdom : Animalia

Phylum : Coelenterata

Class : Anthozoa

Ordo : Scleractinian

Sub Ordo : Fungiina

Familia : Fungiidae

Genus : *Heliofungia*

Species : *Heliofungia actiniformis*



Gambar 1. Morfologi Umum Karang *H. actiniformis*, terlihat tentakel menonjol dari badan karang (Anonim, 2006).

II.1.2 Ekologi Karang

Karang bersimbiosis dengan ganggang zooxantela di dalam sel gastrodermal dimana karang memberikan perlindungan bagi ganggang untuk melakukan fotosintesis. Sementara karang memproduksi gas asam-arang yang merupakan hasil pernapasannya dan menghasilkan metabolisme seperti nitrat, dan fosfat (Barnes., 1987; Barnes dan Hughes, 1999; Lalli dan Parsons, 1995; Levinton, 1995; Sumich, 1996).

Karang pembentuk terumbu hanya dapat tumbuh dengan baik pada daerah-daerah tertentu seperti pulau-pulau yang sedikit mengalami proses sedimentasi. Pertumbuhan dan perkembangan karang sangat baik mulai dari kedalaman 2- 30 m.. Sirkulasi arus yang baik dan rendahnya sedimentasi merupakan andil yang baik bagi tumbuh dan berkembangnya terumbu karang secara optimal (Suharsono, 1998).

Untuk dapat membentuk terumbu karang memerlukan persyaratan hidup tertentu, seperti cahaya, suhu, salinitas, kejernihan air, arus, dan substrat (Nontji, 1988). Cahaya sangat diperlukan untuk proses fotosintesis dari zooxanthella (simbion karang) yang produknya kemudian ditransfer ke hewan karang yang menjadi inangnya. Tanpa cahaya yang cukup, maka laju fotosintesis akan berkurang dan kemudian mengurangi kemampuan karang untuk membentuk kerangka. Titik kompensasi untuk karang nampaknya merupakan kedalaman dimana intensitas cahaya berkurang antara 15-20% dari intensitas cahaya permukaan (Nybakken, 1988).

II.2. Uraian Khusus

II.2.1 Biologi Reproduksi Karang

Pengetahuan tentang reproduksi seksual pada karang telah meningkat setelah ditemukannya masa spawning di *Great Barier Reef* (GBR) dan Wilayah Indo Pasifik lainnya. Mayoritas karang *scleractinian* adalah hermaprodit dengan suatu siklus gametogenesis tahunan. Siklus reproduksi seksual dari *scleractinian* telah dihubungkan dengan faktor

lingkungan. Beberapa studi menyatakan bahwa variasi musiman dalam suhu laut atau fotoperiodik akan menjadi faktor lingkungan yang utama untuk mengontrol siklus reproduksi tahunan. Sementara yang lain mengindikasikan bahwa suhu laut bukan merupakan faktor utama untuk mengotrol waktu reproduksi. Periode *spawning* dari beberapa spesies sering bervariasi sesuai dengan lokasinya. Perbandingan lokasi dari periode *spawning* dari beberapa spesies karang yang sama menjadikan perbedaan respon spesies terhadap kondisi yang bervariasi (Fan & Dai, 1998).

Penentuan tipe seksualitas karang dilakukan menurut petunjuk Richmond & Hunter (1990), Harrison & Wallace (1990), dan Richmond (1997), yaitu:

a. Gonokorik yaitu spesies karang (berkoloni atau soliter) yang memproduksi hanya gamet jantan atau gamet betina.
b. Hermafrodit yaitu spesies karang (berkoloni atau soliter) yang menghasilkan baik gamet jantan atau gamet betina selama hidupnya. Spesies ini memiliki ovum dan sperma yang berkembang dalam mesentri yang sama (umumnya pada Faviidae dan Mussidae), pada mesentri yang berbeda dalam polip yang berbeda dalam koloni yang sama pada (*Cladopsammia rolandi*), atau pada waktu yang berbeda dalam koloni yang sama (*Stylophora pistillata*).

Menurut Szmant (1986), pola reproduksi karang dapat dikelompokkan ke dalam empat golongan, yaitu :

a. Hermaprodit yang diikuti dengan *broadcast spawning* (*hermaphrodit broadcast spawning).*
b. Hermaprodit yang diikuti dengan *brooding (hermaphrodit brooding)*.
c. Gonokorik yang diikuti dengan *broadcast spawning (gonokhorik broadcast spawning).*
d. Gonokorik yang diikuti dengan *brooding* (*gonokhorik brooding*).

Pola-pola umum yang sama terhadap spesies-spesies di Wilayah Indo-Pasifik (Harriot, 1983). Spesies-spesies tersebut dikenal mengikuti pola (a) dan (c), di bawah kondisi lingkungan yang menguntungkan dan ukuran koloni yang lebih besar (diameter lebih besar dari 30 cm) yang secara tidak langsung menunjukkan spesies tersebut berumur panjang,

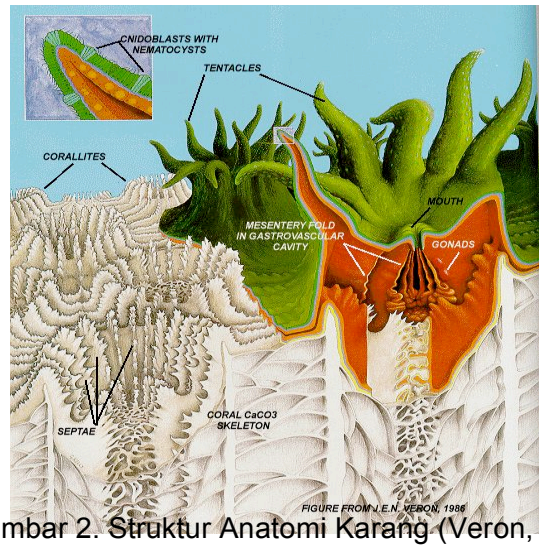
sedangkan spesies-spesies yang mengikuti pola (b) dan (d) adalah secara umum berukuran kecil yang secara tidak langsung menyatakan adanya penurunan pertumbuhan dengan peningkatan umur dan atau kematian induk yang tinggi (Szmant, 1986). Selanjutnya dikatakan bahwa ciri-ciri *brooding* adalah berhubungan dengan ukuran koloni yang kecil dengan banyak siklus reproduksi dalam setahun dan merupakan salah satu bentuk strategi reproduksi pada karang untuk efisiensi dan peningkatan reproduksi baik pada spesies hermaprodit atau gonokorik. Strategi ini juga dapat menghindari tingginya kematian larva pada masa planktonik dan meningkatkan kesempatan larva untuk menemukan substrat yang cocok.

Reproduksi seksual *scleractinian* tidak dapat ditentukan melalui karakteristik eksternal, tetapi dapat diidentifikasi ketika gonadnya matang. Testis secara normal berwarna putih, sedang ovarium berwarna coklat (*Charyophilla smithii, Paracyathus steansii)*, pink (*Farites abdita*), merah (*Leptoria phrygia*), orange (spesies *Lobophila*), atau biru (*Favia favus*). ”*Brooding coral”* juga dapat diidentifikasi ketika embrio diamati di dalam *coelenteron* atau dalam tentakel sebagaimana yang telah dilaporkan pada beberapa kasus (Lacaze-Duthiers 1873; Duerden 1902). Jenis kelamin pada karang terdiri atas gonokorik dan hermaprodit (Richmond & Hunter 1990, Horrison & Wallace 1990, Richmond 1997). Dari 210 spesies yang diteliti sebanyak 142 spesies yang tergolong hermaprodit simultan, yaitu suatu individu yang dapat menghasilkan gamet betina dan gamet jantan dalam waktu yang bersamaan (Richmond & Hunter 1990).

Sebagian besar karang yang berkoloni adalah hermaprodit seperti *Astroides calycularis, Favia fragum, Favia fallida. Cladosammia rolandi* adalah satu-satunya koloni karang yang hermafrodit tetapi polipnya gonokorik. Banyak koloni karang memiliki koloni gonokorik sejati dimana semua polip bisa jantan atau betina misalnya *Astrangia danae, Porites andrewesi, Goniopora queenslandiae decima.* Karang yang soliter juga gonokorik contoh *Ballanophyllia elegans, Caryophyllia smithi* atau yang hermaprodit adalah *H. actiniformis* (Fadlallah, 1983).

Studi terdahulu (Lacaze-Duthiers 1873: Abe 1937) mengindikasikan bahwa *brooding* terjadi secara bebas di dalam *coelenteron*, juga menyatakan bahwa fertilisasi serta perkembangan oosit yang bebas terjadi dalam rongga *coleonteron*. Fertilisasi dan awal tahap perkembangan embrionik terjadi dalam lapisan mesoglea pada gonad yang berlokasi pada septa polip Ketebalan jaringan septa yang mengelilingi oosit dan embrio berfungsi melindungi dan memberi nurtisi untuk endoderm.

Umumnya ovarium dan testis karang berkembang pada mesentri didalam mesoglea atau gastrodermis (Harisson & Wallacea 1990). Gonad dari karang *Acropora nobilis* dan *Pocillopora verrucosa* teramati berkembang dalam lapisan mesoglea (Rani, 2004). Gonad-gonad tersebut kemudian bergerak menuju mulut ketika memasuki tahap pematangan. Gonad pada karang *A. nobilis* memanjang dan menyerupai tabung serta tergantung di dalam rongga gastrovaskuler di bawah mulut. Sedangkan *P. verrucosa* terlihat berbentuk bulat. Sel telur dan testis dari *A. nobilis* selalu didapatkan dalam polip yang sama dengan struktur yang mirip dengan sembilan spesies simpatrik dari genus *Acropora* yang ditemukan oleh Wallacea (1985) di Australia Timur. Sedangkan pada *P. verrucosa*, sel telur dan testis secara terpisah atau bersama-sama dalam satu polip yang sama dan memperlihatkan ukuran atau volume testis yang lebih besar dari volume telur. Hal ini juga teramati pada *P. damicornis* dan *P. elegans* di bagian timur pasifik (Glynn *et all.*1991).



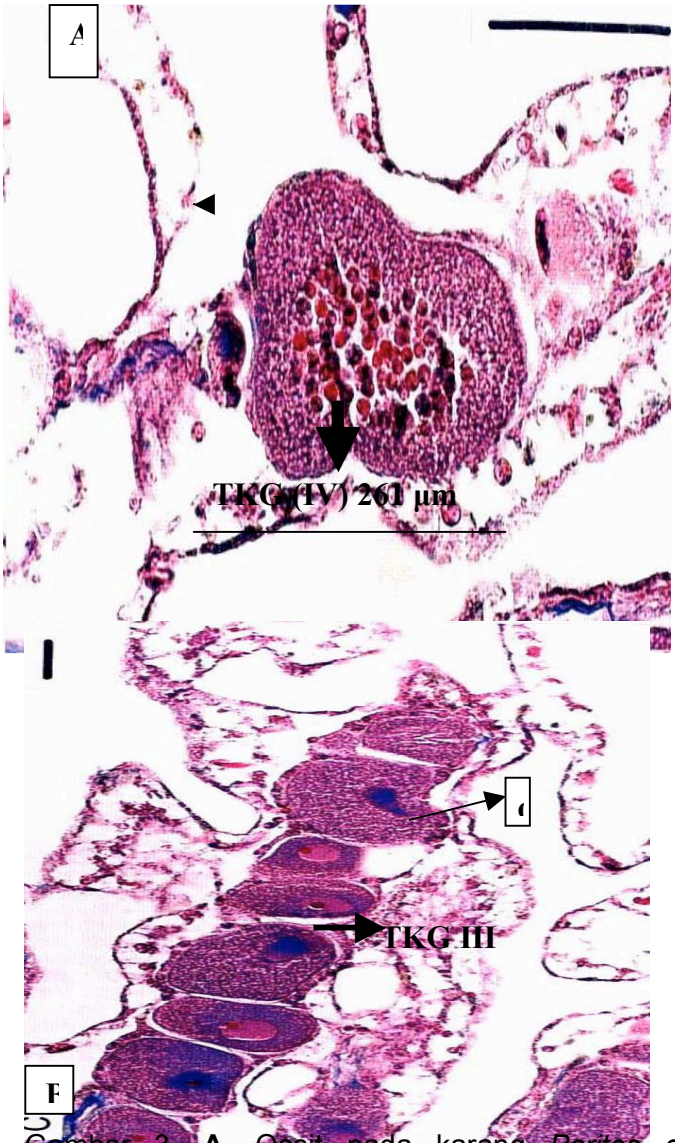
Gambar 2. Struktur Anatomi Karang (Veron, 1986)

II.2.2 Perkembangan Gonad

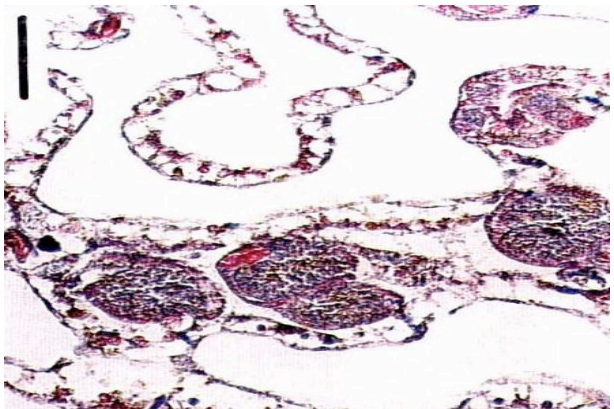
Perkembangan gonad dari *A. nobilis* dan *P. verrucosa* menurut (Rani, 2004) bahwa karakter dari setiap tahapan perkembangan sperma dan ovum dari kedua spesies ini memiliki tingkat kemiripan yang sangat tinggi. Perbedaan yang ditemukan hanya terletak pada ukuran dan jumlah lobus dalam testis, dimana tahap pertama dari perkembangan sperma yaitu testis membentuk "dambel" dengan dinding sel yang tebal yang kemudian berubah menjadi bentuk lonjong dengan batas antara sel sperma sudah terlihat pada tahap II. Pada tahap II ini testis terlihat berwarna kebiru-biruan. Dalam tahap perkembangan selanjutnya karakternya dapat dibedakan berdasarkan ukuran volume testis dan karakter dari sitoplasma dan inti sel sperma. Testis meningkat dalam ukuran bulat lonjong atau tidak beraturan dengan volume sitoplasma yang bertambah dan inti spermatosit yang memadat ketika berada dalam tahap III. Pada tahap akhir volume sitoplasma berkurang dan terbentuk ekor spermatosit. Spermatozoa tiba dalam lumen dan seringkali membentuk "buket". Sedang perkembangan sel telur pada tahap awal dapat dibedakan dengan jelas berdasarkan bentuk dan ukuran selnya. Pada tahap I, sel telur berbentuk bulat lonjong dengan dinding sel yang belum terbentuk dan ukuran oosit yang relatif tebal dan berbentuk bulat. Sedangkan tahap selanjutnya dapat dibedakan dengan berdasarkan posisi inti telur dan meningkatnya butiran lemak. Pada tahap III, butiran lemak memadat dan meningkat dalam ukuran dan posisi inti berada di bagian tengah oosit dan ketika masuk dalam tahap matang inti oosit bergerak ke tepi dan kadang-kadang memperlihatkan bentuk yang berlekuk seperti pelana dengan butiran lemak yang semakin jelas.

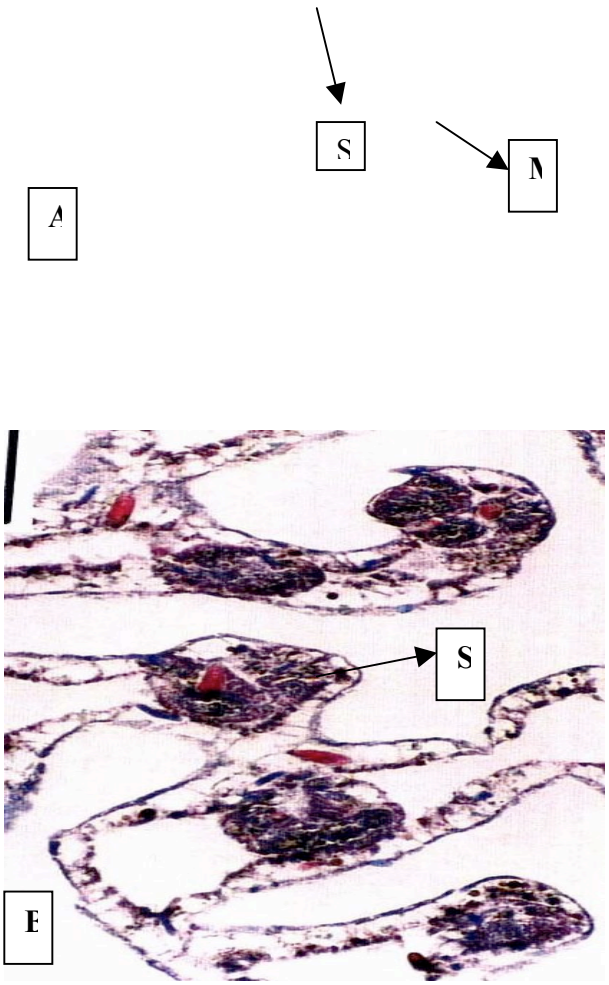
Sementara studi perkembangan gonad dari karang *Porites evermanni* yang dilaporkan (Neves, 1998) menjelaskan bahwa karang *P. evermanni* memiliki mode reproduksi gonokorik *broadcast spawner.* Data yang berhubungan dengan perilaku reproduktif dan struktur gonad dari *P. evermanni* sangat langka (Richmond dan Hunter, 1990). Dalam empat sampel gonad ditemukan tiga jantan dan satu betina yang telah diamati sepanjang musim panas dari 1997. Tahap pendewasaan juga terjadi dalam filamen mesentri. Analisis histologi menunjukkan adanya droplet lipid dan zooxantela. Zooxantela

pada awalnya terdapat dalam endoderm menutupi oosit kemudian menyatu dalam sitoplasma. Kebanyakan sperma belum poten, dan biasanya mempunyai satu atau dua nematosit holotricus. Ovum dan testis berukuran 143 µm dan 261 µm.



Gambar 3. **A.** Oosit pada karang *Porites evermanni* dengan Tingkat Perkembangan Gonad IV dengan diameter 261 µm. **B**. Oosit dengan Tingkat Perkembangan Gonad III dengan diameter 143 µm (Neves, 1998).





Gambar 4. **A** dan **B** sperma karang *Porites evermanni* yang belum matang dengan skala 100µm. S (sperma), M (mesentri) (Neves, 1998).

BAB III

METODE PENELITIAN

◄

III.1 Alat

Alat yang digunakan selama penelitian ini adalah peralatan *scuba diving*, gunting, mistar, martil, objek gelas, *cover glass*, botol sampel, box *glass objek*, foto mikroskop, *cutter*, gelas piala, histoembedder, mikroskop, mikrotom, pinset, toples, *tissue cassette* dan *deckel*, scapel, pensil.

III.2 Bahan

Bahan yang digunakan dalam penelitian ini adalah sampel karang *Heliofungia actiniformis,* alkohol 70%,80%,96%, 100%, Aquadest, Entelan, Formalin 5%, HCl, Haematoxcilin, Alkohol asam, Eosin, Larutan Xilol, Paraffin, kertas label.

III. 3 Metode Kerja

III.3.1 Metode pengambilan sampel

Pengambilan sampel dilakukan berdasarkan siklus bulan atau tahun Hijriyah, yaitu pada fase bulan baru, setengah menuju penuh, bulan penuh (bulan purnama), bulan setengah menuju bulan baru. Pengambilan sampel ini dilakukan dengan menggunakan alat SCUBA (*Self Containing Underwater Buoyancy Apparatus*) pada kisaran kedalaman 8 – 17 meter. Sampel karang yang diambil berukuran ≥ 10 cm (Abe, 1937). Sampel tersebut dibelah menjadi 2 bagian, satu bagian diambil dan bagian yang lain dibiarkan di tempat semula. Sampel kemudian dimasukkan kedalam botol plastik dan kemudian diawetkan dengan menggunakan formalin 5% (formalin dilarutkan dengan air laut).

III.3.2 Pemisahan Skeleton dengan Jaringan (*Dekalsifikasi*)

Sebelum melakukan pengerjaan secara histologi, sampel terlebih dahulu dipisahkan dari skeletonnya. Pemisahan tersebut dilakukan dengan menggunakan Asam Chlorida (HCl) dengan konsentrasi 12% (HCl dicampur dengan aguades) (Harii *et al*., 2001).

Sampel yang telah diawetkan di dalam formalin 5% sebelum diluruhkan terlebih dahulu dicuci dengan air tawar mengalir hingga formalin lepas dari permukaan karang ditandai dengan bau yang telah hilang dari sampel tersebut.

Setelah itu, sampel dimasukkan ke dalam larutan HCl 12% dan dibiarkan selama ± 24 jam hingga seluruh kapurnya $CaCO_3$ luruh ke dalam HCl.

Jaringan kemudian diangkat dari larutan dan dicuci dengan air mengalir hingga bersih. Setelah itu, jaringan karang tersebut dimasukkan ke dalam botol yang berisi alkohol 70% selama 2 x 24 jam.

III.3.3 Analisis Histologi

Penyiapan sediaan histologi mengikuti teknik jaringan standar yakni dengan menngunakan metode parafin dengan fiksatif formalin dan pewarnaan (HE) Haematoxylin Eosin (Humason, 1962; Wallace, 1985; Kiernan, 1990; Glynn *et al*., 1991, 1994). Proses dehidrasi dengan menggunakan alkohol bertingkat (70%-80%,96%,100%). *Clearing* menggunakan larutan Xylol serta diinfiltrasi dengan parafin cair titik leleh 56-58°C. Sampel tersebut selanjutnya ditanam dalam blok parafin (*embedding*). Jaringan dipotong dengan mikrotom setebal 6 μm. Pewarnaan (*staining*) dengan *Haematoxylin Eosin* (HE). Untuk mengamati perkembangan gonad digunakan mikroskop cahaya dengan pembesaran 40x dan pengambilan gambar dilakukan di bawah fotomikroskop dengan menggunakan film ASA 200.

Tahap perkembangan gonad diidentifikasi berdasarkan karakter dari ukuran sel dan bentuk morfologinya serta karakter warna yang dihasilkan dari pewarnaan HE (Hematoxylin-Eosin) yang digunakan. Selain itu keberadaan inti dan kandungan sel sperma juga dapat digunakan untuk mengidentifikasi karakter tersebut. Identifikasi dari karakter setiap perkembangan sel sperma dan telur telah diidentifikasi juga dengan berdasarkan modifikasi dari petunjuk (Glynn *et al*. 1991, 1994).

III.3.4 Analisis data

Data mengenai seksualitas, model reproduksi dan tingkat perkembangan gonad disajikan dalam bentuk gambar histologi fotomikrograf dan dianalisis secara deskriptif.

Keterkaitan antara fase bulan dengan tingkat perkembangan gonad dianalisis secara deskriptif dengan bantuan grafik dengan fase bulan sebagai sumbu X dan jumlah telur sebagai sumbu Y.

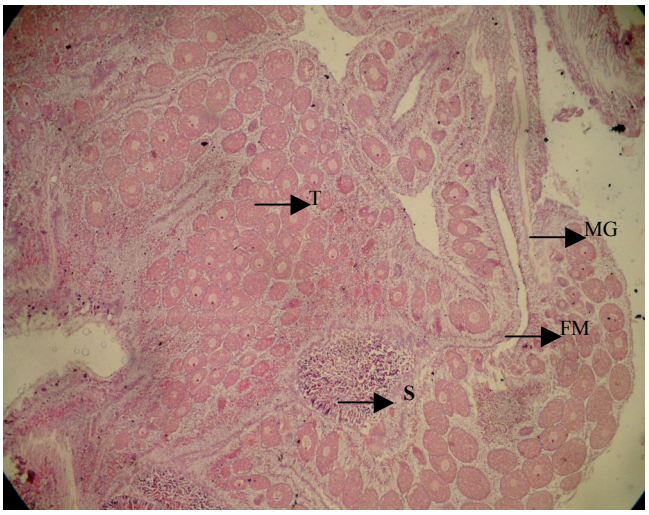
BAB IV

HASIL DAN PEMBAHASAN

IV.1 Seksualitas dan Mode Reproduksi

Hasil yang diperoleh melalui studi histologi menunjukkan bahwa karang *H actiniformis* memiliki tipe seksualitas hermaprodit. Fenomena ini dapat dibuktikan dengan kehadiran ovum dan testis dalam polip yang sama (**Gambar 5),** namun demikian ovum dan testis berkembang dalam filamen mesentri yang berbeda**.**

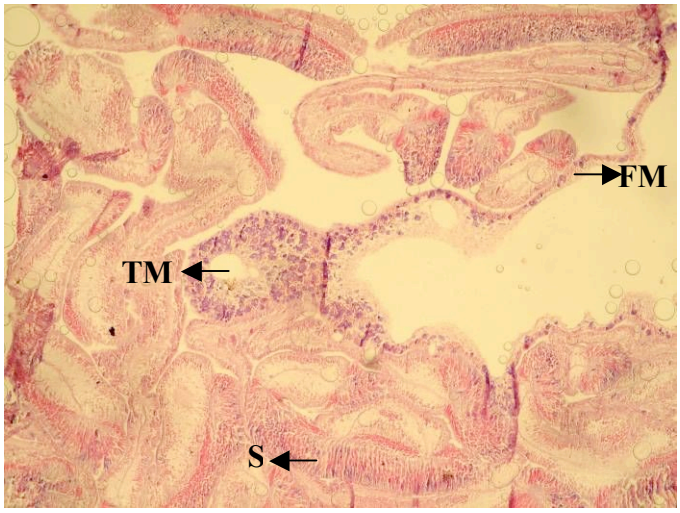
Fenomena yang sama juga teramati pada *Pocillopora verrucosa* (Rani, 2004)**.** Berdasarkan (Harisson & Wallacea 1990) umumnya ovarium dan testis karang berkembang pada mesentri didalam mesoglea atau gastrodermis.



Gambar 5. Penampang melintang gonad karang *Heliofungia actiniformis.* T = Telur, FM = Filamen Mesentri, S = Sperma, MG = Mesoglea. **Data sampling 03 Juli 2006 (HE ; 400X).**

Sebagian besar karang yang berkoloni adalah hermaprodit seperti *Astroides calycularis, Favia fragum, Favia fallida. Cladosammia rolandi* adalah satu-satunya koloni karang yang hermaprodit tetapi polipnya gonokorik. Banyak koloni karang memiliki koloni gonokorik sejati dimana semua polip bisa jantan atau betina seperti *Astrangia danae, Porites andrewesi, Goniopora queenslandiae decima.* Karang soliter yang gonokorik ditemukan pada *Ballanophyllia elegans* dan *Caryophyllia smithi* sedangkan yang soliter dan hermaprodit seperti *H. actiniformis* (Fadlallah, 1983).

Adanya testis yang kosong atau dipijahkan menjadi indikator bahwa karang *H actiniformis* tergolong hermaprodit *spawning* (**Gambar 6)** yakni spesies yang melepaskan gametnya (telur dan sperma) ke dalam kolom air dan selanjutnya terjadi fertilisasi eksternal dan kemudian terjadi perkembangan embrio.



Gambar 6. **TM** = Kantong yang sudah kosong (sperma sudah dipijahkan), **FM** = Filamen Mesentri, **S** = Sperma. Data sampling tgl 14 September 2006**(HE; 400X).**

Mode reproduksi karang *H. actiniformis* yang bersifat pemijah yang hermaprodit merupakan tipe umum dari karang *scleractinian* (Harrison & wallacea 1990, Richmond &

Hunter 1990, Richmond 1997). Pola reproduksi ini umum ditemukan diwilayah Indo-Fasifik (Harriot, 1983).

IV.2 Karakter Perkembangan Gonad Karang *H. actiniformis*

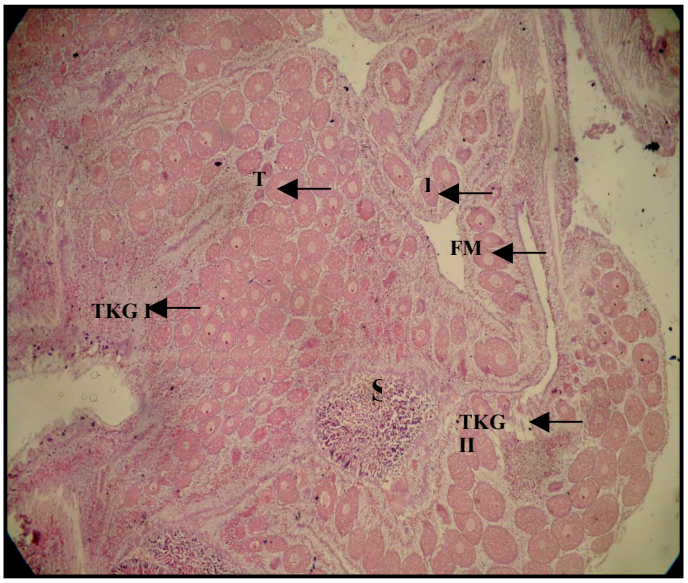
Perkembangan telur dari karang *H. actiniformis* dapat disajikan Tabel 1 berikut:

Tabel 1. Tahap perkembangan telur karang *H. Actiniformis*

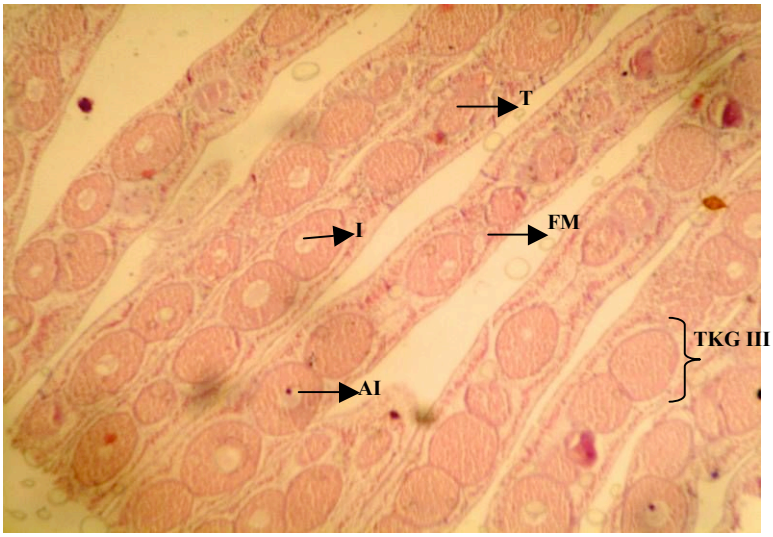
Tahap	Karakteristik Telur	Rata-rata Diameter Telur (µm)	Kisaran Diamater Telur	Keterangan
I	Bentuk bulat kecil,inti belum terlihat jelas,berkumpul dalam mesoglea	8 µm	4-12 µm	**Gambar 7**
II	Bentuk bulat lonjong terdapat inti dan anak inti pada bagian tengah sel, masih berkumpul dalam mesoglea.	12 µm	10-15 µm	**Gambar 7**
III	Bentuk bulat lonjong,inti dan anak inti semakin besar	15 µm	11-20 µm	**Gambar 8**
IV	Bentuk selnya semakin lonjong dan padat,inti terlihat jelas dan bergerak ke tepi sel	23 µm	17-30 µm	**Gambar 9**

Tahap kematangan gonad dapat diidentifikasi melalui ukuran sel, morfologi dan karakter warna yang dihasilkan oleh pewarna HE (Haematoxylin-Eosin). Karakter dari tahapan kematangan gonad diidentifikasi berdasarkan hasil modifikasi petunjuk Glynn *et al.* (1991,1994). Berdasarkan hasil pengamatan bahwa pada **Tahap I,** sel telur berkumpul dalam mesoglea dengan bentuk bulat kecil dan berwarna merah dengan diameter 4 µm. **Tahap II**, ditandai dengan ukuran sel telur yang semakin bertambah yaitu sekitar 15 µm, dimana pada bagian tengahnya terdapat inti sedangkan anak inti belum terlihat jelas. **Tahap III,** bentuk sel semakin bulat lonjong dengan ukuran sel 20 µm, sel terlihat berwarna merah, inti sel terdapat pada bagian tengah dan selnya semakin padat. **Tahap IV,** bentuk sel

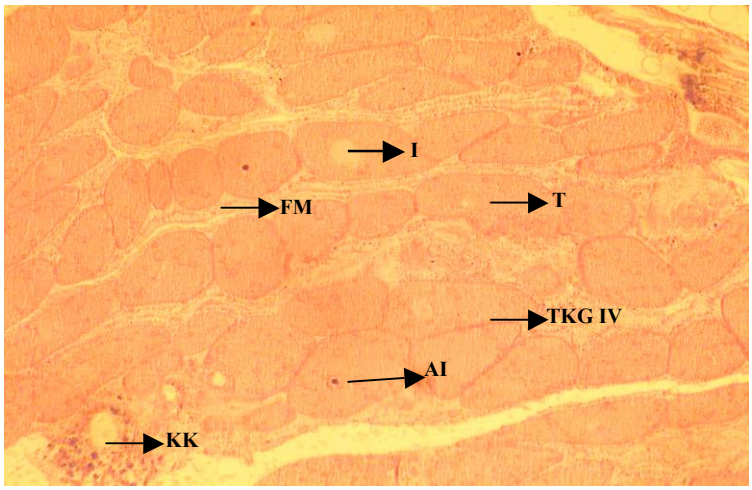
bertambah padat dan ukuran diameternya semakin bertambah dalam tahapan ini inti terlihat sangat jelas.



Gambar 7. Penampang melintang gonad *H. aciniformis* S = Sperma, T = Telur, FM = Filamen Mesentri, I = Inti sel (nukleus), TKG I = Oosit dengan **Tingkat Kematangan Gonad I** skala 4 µm **TKG II** = Oosit dengan **Tingkat Kematangan Gonad II** dengan skala 15 µm. (**data sampling tanggal 03 Juli 2006**)(HE ; 400 X).



Gambar 8. Oosit dengan TKG III (**III)** dengan skala 20 µm. T = Telur, FM = Filamen Mesentri, I = Inti sel (nukleus), AI = Anak Inti (nukleolus) (**data sampling tanggal 23 Agustus 2006**) (HE ; 400X).



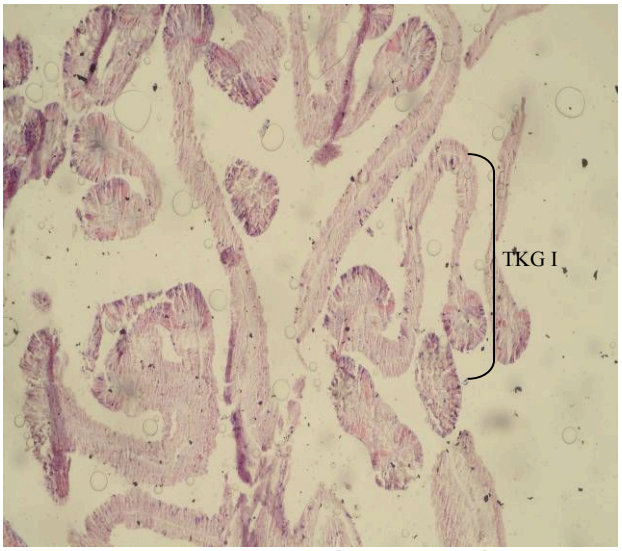
Gambar 9. **O**osit dengan Tingkat Kematangan Gonad (TKG) IV dengan skala 30 µm. T = Telur, FM = Filamen Mesentri, I = Inti sel (nukleus), AI = Anak Inti (nukleolus) KK = Kantong kosong yang sudah dipijahkan (telur yang sudah *spawning*) (**data sampling tanggal 14 September 2006**) (HE ; 400X).

Tabel 4. Tahap perkembangan **sperma** karang *H. Actiniformis*

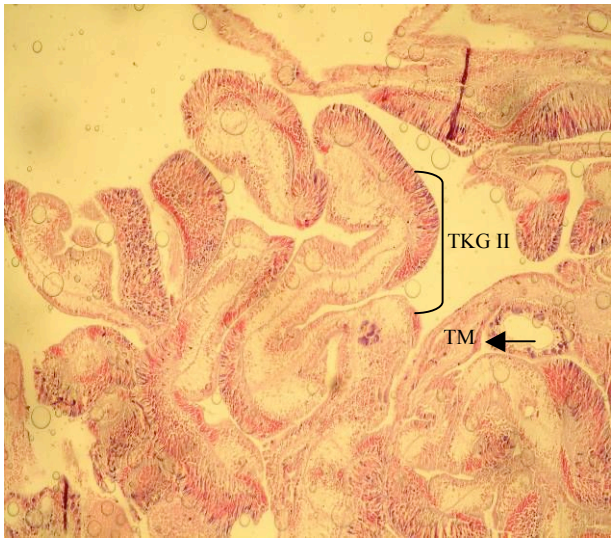
Tahap	Karakteristik Testis	Rata-rata Diameter Testis (µm)	Kisaran Diameter Testis (µm)	Keterangan
I	Berbentuk dambel, berwarna ungu	40 µm	34-47 µm	**Gambar 10**
II	Berbentuk bulat lonjong dan padat dengan diameter yang semakin bertambah	51 µm	51-57 µm	**Gambar 11**
III	Diameter testis semakin bertambah dan memadat yang terwarnai dengan warna kebiru-biruan	57 µm	54-61 µm	**Gambar 12**
IV	Ukuran testis semakin besar, berwarna biru pekat keunguan	68 µm	67-70 µm	**Gambar 13**

Untuk tahap perkembangan sperma pada **Tahap I,** sel berasal dari germinal sel yang akan berkembang menjadi testis, berbentuk dambel dan berwarna ungu kebiru-biruan. **Tahap II,** berbentuk bulat lonjong diikuti dengan bertambahnya ukuran diameter sperma. **Tahap III,** volume sperma semakin meningkat dan padat dan berwarna biru pekat. Memasuki **Tahap IV**, ukuran sel semakin meningkat berbentuk bulat padat dengan warna biru pekat. Berdasarkan hasil analisis histologi dapat dijelaskan bahwa sperma lebih cepat matang jika dibandingkan dengan sel telur.

Berdasarkan hasil data yang diperoleh Karang *H. actiniformis* kemungkinan memijah pada fase bulan gelap yang ditandai dengan adanya ovum dan sperma yang memasuki TKG IV pada fase tersebut.



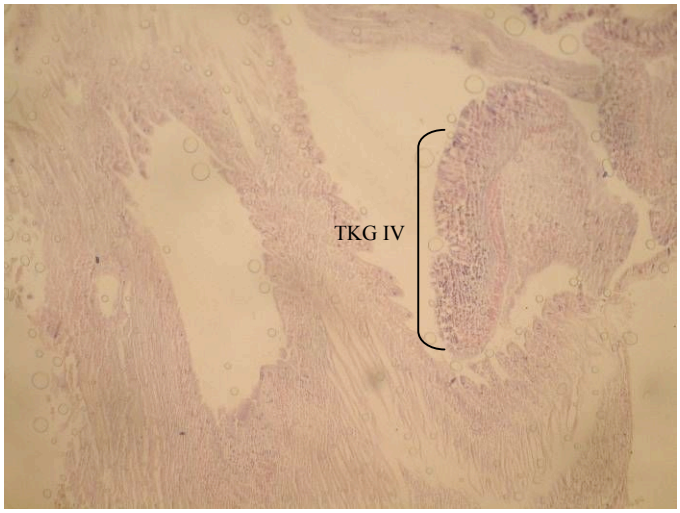
Gambar 10. Tingkat Kematangan Gonad (**TKG I)** dengan skala 47 µm (**data sampling tanggal 8 Agustus 2006**) (HE ;400X).



Gambar 11. Tingkat Kematangan Sperma II dengan skala 57 µm**. TM =** kantong kosong (testis dengan sperma yang *spawning*) (**data sampling tanggal 14 September 2006**) (HE ; 400X).



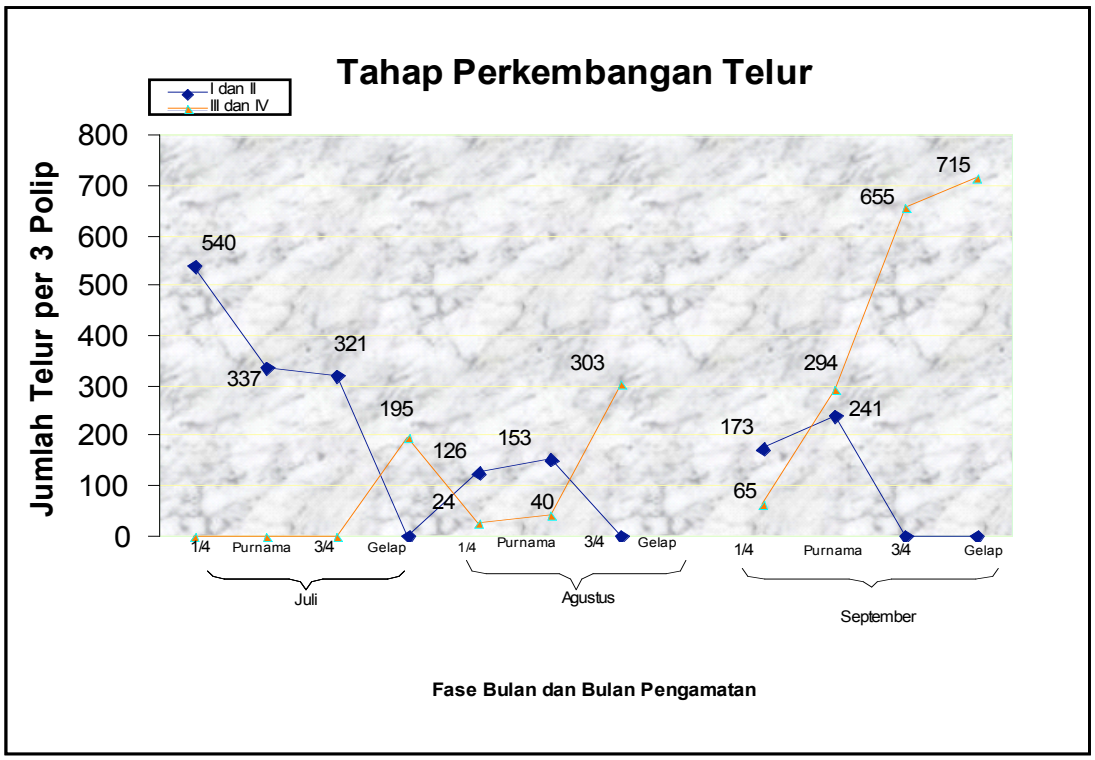
Gambar 12 ad (TKG III) dengan skala 60 µm. (**data sampling tanggal 23 Agustus 2006)** (HE; 400X).



Gambar 13. Tingkat kematangan gonad IV dengan skala 70 µm. **Data sampling tanggal 14 Juli 2006**. **(HE; 400X).**

IV.3 Keterkaitan Fase Bulan Hijriah Dengan Tingkat Perkembangan Gonad

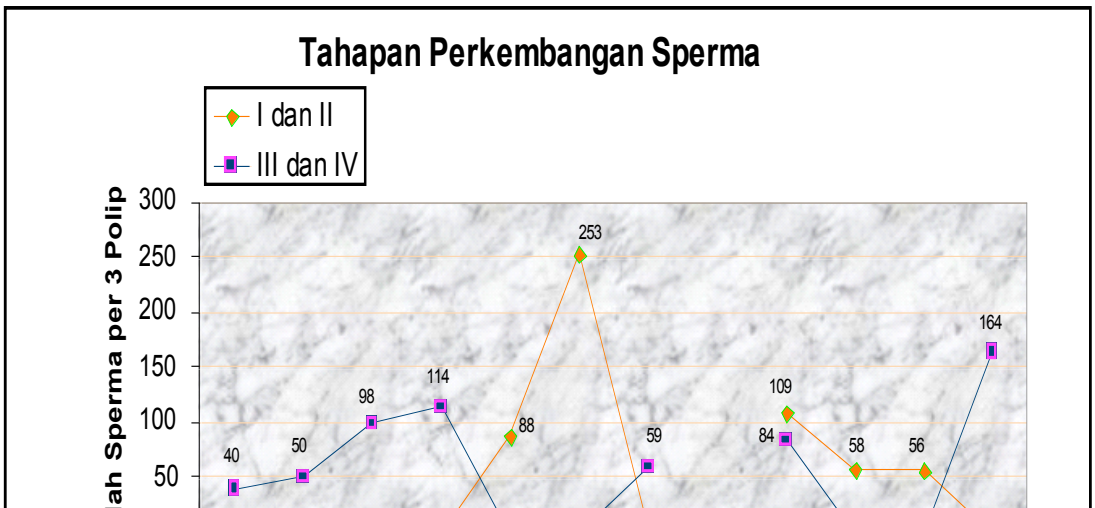
Keterkaitan fase bulan dengan tingkat perkembangan gonad dapat dilihat pada **Gambar 14 -15** dan **Lampiran 1-6** :



Gambar 14. Sebaran jumlah telur menurut fase bulan selama tiga bulan pengambilan sampel (fase bulan gelap pada bulan Agustus tidak dilakukan pengambilan sampel).

Berdasarkan data bahwa jumlah telur pada TKG I dan II karang *H. actiniformis* cenderung menurun pada fase ¼ sampai fase gelap di bulan Juli, dan mengalami peningkatan pada fase bulan purnama di bulan Agustus dan September. Pada fase ¼ jarang ditemukan telur yang berada pada TKG III dan IV bahkan di bulan Juli tidak ditemukan sama sekali. Pada fase bulan gelap di setiap bulan selama penelitian ditemukan TKG III dan IV sebaliknya tidak ditemukan TKG I dan II. Fenomena ini mengindikasikan bahwa karang *H. actiniformis* melakukan pemijahan pada fase bulan gelap.

Fase bulan gelap bulan Agustus tidak dilakukan pengambilan sampel yang diakibatkan kondisi dan cuaca yang tidak mendukung.



Gambar 15. Sebaran jumlah sperma menurut fase bulan selama tiga bulan pengambilan sampel (fase bulan gelap pada bulan Agustus tidak dilakukan pengambilan sampel).

Perkembangan Sperma karang *H. actiniformis* tahap I dan II pada bulan Juli cenderung meningkat dari fase bulan ¼ sampai fase bulan gelap selanjutnya menurun kembali pada fase bulan ¼ dibulan Agustus. Dari grafik dapat dilihat bahwa jumlah testis TKG I dan II tertinggi pada fase bulan gelap. Pada fase ¼ sampai fase bulan gelap dibulan Juli tidak ditemukan sperma yang berada pada TKG III dan IV. Sedangkan jumlah testis TKG III dan IV cenderung meningkat pada fase bulan gelap selama tiga siklus bulan. Hal ini juga mengindikasikan bahwa karang ini *spawning* difase bulan gelap. Sebagai bahan perbandingan telah dicatat beberapa jenis karang yang memijah pada fase bulan gelap terjadi pada karang *A. nobilis* dan *P. verrucosa* di Pulau Barranglompo, Makassar, Sulawesi Selatan (Rani, 2004). Demikian juga pada *Porites lobata* di Pulau Cano (Costa Rica) dan Pulau Uva (Panama) yang diindikasikan dengan peningkatan perkembangan gonad di sekitar bulan purnama dan bulan gelap (Glynn *et al,* 1994). Populasi karang *Pontipora compressa* di Hawaii juga teramati memijah pada fase bulan purnama dan bulan gelap (Hodgson, 1985).

Fase bulan merupakan suatu isyarat untuk pemijahan secara sinkron . Pemijahan pada fase bulan gelap akan meminimalkan predasi dari predator yang menggunakan ketajaman matanya seperti jenis-jenis ikan pemakan plankton (*Abudelfduf*, *Neopomacentrus* dan *Pomacentrus*) (Babcock *et al*, 1986).

Pada kebanyakan hewan laut, siklus bulan mungkin memacu waktu pematangan gonad (Norton 1981, Philips *et al,* 1990), demikian pula pada karang (Wallcea 1985, Glynn *et al,* 1991, McGuire 1998). Selain itu fase bulan juga mengatur waktu pelepasan hasil

reproduksi, seperti pelepasan larva dari *Pocillopora damicormis* di Hawai (Richmond & Jokiel 1984, Jokiel *et al,* 1985) dan *Porites astreoides* di Teluk Florida (McGuire, 1998). Menurut Babcock *et al* (1994), karang menggunakan cahaya bulan sebagai signal lingkungan yang tepat untuk memprediksi pola pasang surut dalam pemijahan bersama.

BAB V

PENUTUP

V. 1 Kesimpulan

Berdasarkan hasil yang diperoleh maka dapat disimpulkan bahwa:

1. Karang *Heliofungia actiniformis* memiliki tipe seksualitas *hermafrodit* dengan mode reproduksi *hermafrodit spawning.*
2. Tingkat kematangan gonad karang *H. actiniformis* dicirikan dengan meningkatnya diameter ketika menuju ke kematangan gonad termasuk perubahan dari bulat ke bulat lonjong ketika matang.
3. Karang *H. actiniformis* melakukan pemijahan pada fase bulan gelap yang ditandai dengan meningkatnya jumlah telur dan sperma (Tingkat Kematangan Gonad III dan IV) pada fase bulan gelap dan menurun pada fase bulan ¼.

V.2 Saran

Kurangnya data dan informasi mengenai biologi reproduksi karang hias maka kami menyarankan untuk melakukan penelitian lanjutan mengenai biologi reproduksi karang hias pada spesies yang berbeda dalam kaitannya dengan perkembangan gonad untuk pengembangan budidaya karang hias.

DAFTAR PUSTAKA

Abe, N.1937. *Post Larva Development of the Coral Fungia actiniformis* var. *Palawensis Doderlein.* Palao Trop Biol Stat Study 1:73-93.

Anonim., 2006. http://library.thinkquest.org/26153/marine/cnida.htm. (diakses tgl 24 September 2006).

Barnes, R.D. 1987. *Invertebrate Zoology;* Fifth Edition. Fort Worth, TX: Harcourt Brace Jovanovich College Publishers. pp. 92-96, 127-134, 149-162.

Barnes, R.S.K. and Hughes R.N. 1999. *An Introduction to Marine Ecology*; third edition. Oxford, UK: Blackwell Science Ltd. pp. 117-141.

Babcok RC, Bull GD, Harrison PL, Heyward AJ, Oliver JK, Wallace CC, Willis BL. 1986. *Synchronous spawings of 105 scleractinian coral species on the great barrier Reef*. Mar Biol 90: 379-394.

Babcock RC, Wills BL, Simpson CJ. 1994. *Predictable and unpredictable spawning events*. Invert Repro Develop 22: 1-3

Duerden, J. E. 1902. *West Indian Madreporarian Polyps*. *Mem.Nat. Acad.Sci.*

Fan., Dai. 1998. *Sexual Reproduksi of The Skleractinian Coral Merulina ampliata In Southern Taiwan.*

Fadlallah, Y.H. 1983. *Population Dynamics and Life History of a Solitary Coral Balanophyllia elegans,* from central California.

Giese, A.C and Pearse, J.S.1974. *Introduction General Principles In Reproduction of Marine Invertebrates, vol.I*. Academic Press,New York.

Glynn, P.W., Gassman N.J., Eakin C.M.., Cortes J., Smith D.B., and Guzman H.M. 1991. *Reef Coral Reproduction in the Eastern Pacific*: Costa Rica, Panama, and Galapagos Islands (Ecuador). *I Pocilloporidae*. *Marine Biology* 109: 355–368.

Glynn, P.W., Colley S.B., Eakin C.M., Smith D.B., Cortes J., H.M. Guzman., J.B. Del Rosario, and J.S. Feingnold. 1994. *Reef Coral Reproduction in the Eastern Pacific: Costa Rica, Panama and Galapagos Islands (Ecuador). II Poritidae.* Marine Biology 118: 191–208.

Gardiner, M.S. 1972. *The Biology of Invertebrates. McGraw–Hill Book company*. New York. pp. 759-880.

Goreau, N. I. 1981. *Setting., Survivorship, and Spatial Aggregation in Planula and Juveniles of the Coral Porites porites* (Pallas).

Green EP and Shirley F. 1999. *The Global Trade in Coral. World Conservation Monitoring Centre.* Word Conservation Press, Cambridge UK, 60 pp.

Harrison, P.L, and C.C. Wallace. 1990. *Reproduction, Dispersal and Recruitment of Scleractinian Corals: In Dubinsky (ed). Coral reefs: Ecosystems on the world 25. elsevier.* Amsterdam–Oxford, New York–Tokyo pp. 132–207.

Humason, G.L. 1962. *Animal Tissue Techniques*. W.H. Freeman dan Company, San Francisco and London.

Harriot, V.J. 1983. *Reproductive Seasonality, Settlementy Settlement and Post Settlement of Pocillopora damicornis (Linnaeus)* Aktiva Lizard Island, Great Barrier Reef. *Coral Reefs* 2: 151–157.

Harii, S., M. Omori., H. Yamakawa., Y. Koike. 2001. *Sexual Reproduction And Larval Settlemen of the Zooxanthellate Coral Alveopora japonica* Eguichi *at High Latitudes.* Departement of Aquatic Biosciences Tokyo University of Fisheries.

Jokiel PL, Ito RY, Liu PM. 1985. *Night irradiance and synchronization of lunar release of planula larvae in the reef coral Pocillopora damicormis.* Mar Biol 88: 155-162

Kiernan JA., 1990. *Histological & Histochemical Method***s**: Theory and Practice. Sanfransisco & London: Pergamon Pr.

Lalli, C.M. and T.R. Parsons. 1995. *Biological Oceanography: An Introduction*. Oxford, UK: Butterworth-Heinemann Ltd. pp. 220-233.

Levinton, J.S. 1995. *Marine Biology: Function, Biodiversity, Ecology*. New York: Oxford University Press, Inc. pp. 306-319.

Lacaze-Duthiers H de . 1873. *Development des coralliares. Actinaires a Polypiers.* Arch Zool Exp Gen 2: 269-348.

McConnaughey, B.H., Zottoli, R. 1983. *Pengantar Biologi Laut.* C.V. Mosby Company. London.

Nybakken, 1988. *Biologi Laut Suatu Pendekatan Ekologis*. Gramedia, Jakarta.

Nontji, A. 1988. *Laut Nusantara*, Djambatan, Jakarta.

Norton TA. 1981. *Gamete expulsion and release In Sargassum muticum*. Bot Mar 24: 465-470

Neves, E. 1998. *Histological Analysis of Reproductive Trends of Three Porites Species From Kane Ohe Bay Hawaii.* Departement of Invertebrates. Brazil.

Philips JA, Clayton MN, Maier I, Boland B, Muller Dg. 1990. Sexual reproduction in Dictyota diemensis (Dyctyotales, Phaeophyta) Phycologia 29: 367-379.

Rani, C., 2004. *Reproduksi Karang Sclerectinia Acropora nobilis dan Pocilopora veruccosa di Terumbu Karang Tropis Pulau Barrang Lompo*. Desertasi (S3). Institud Pertanian Bogor. Bogor.

Richmond R.H, and Hunter CL., 1990. *Reproduction and Recruitment in Corals*, Comparisons among the Caribbean, the tropical pacific, and the Red Sea.

Richmond, R.H. 1997. *Reproduction and Recuitment in Corals: Critical links in the persistence of reefs*. in Birkeland, C. (ed.). *Life and death of coral reefs*. Chapmann & Hall. PP 175–197.

Richmond RH, Jokiel PL. 1984. *Lunar periodicity in larva release in the reef coral Pocillopora damicormis at enewetak and Hawaii*. Bull Mar Sci 345 (2): 80-287.

Szmant,A.M.1986*. Reproductive ecology of Carribean reef coral.* Coral Reef 5:43-45.

Sudjana., 1992. *Metode Statistika Edisi ke 5*. Tarsito. Bandung.

Sumich, J.L. 1996. *An Introduction to the Biology of Marine Life*, sixth edition. Dubuque, IA: Wm. C. Brown. pp. 255-269.

Suharsono. 1996. *Jenis-jenis Karang yang Umum Dijumpai di Perairan Indonesia.* Proyek Penelitian dan Pengembangan Daerah Pantai, Pusat Penelitian dan pengembangan Oseanologi. Lembaga Ilmu Pengetahuan Indonesia, Jakarta.

Turgeon, D.D. and R.G. Asch. 2006. *The State of Coral Reef Ecosystems of the United States and Pacific Freely Associated States.* Washington D.C.; NOAA.

Wabnitz, C, Tailor M, Green E and Razak T. 2003. *From Ocean to aquarium.* UNEP-WCMC. Cambridge. UK

Wallace, C.C. 1985. *Reproduction; Recruitment and Fragmentation in Nine Sympatric Species of the Coral Genus Acropora. Marine biology* 88: 112–233.

Veron, J.E.N. 1986. *Coral of Australia and the Indonesia-Pacific August. Robertson. Publish.*

Lampiran 1. Tahap Perkembangan Telur Karang *H. actiniformis* Bulan Juli 2006

Tahap Perkembangan Telur	Bulan ¼ (3 Juli 2006)	Bulan purnama (9 Juli 2006)	Bulan ¾ (14 Juli 2006	Bulan Gelap (23 Juli 2006)
I	378	120	73	0
II	162	217	248	0
Total	**540**	**337**	**321**	**0**
III	0	0	0	35
IV	0	0	0	160
Total	**0**	**0**	**0**	**195**

Lampiran 2. Tahap Perkembangan Telur Karang *Heliofungia actiniformis* Bulan Agustus 2006.

Tahap Perkembangan Telur	Bulan ¼ (8 Agustus 2006)	Bulan purnama (15 Agustus 2006)	Bulan ¾ (23 Agustus 2006)
I	0	0	0
II	126	153	0
Total	**126**	**153**	**0**

III	24	23	0
IV	0	17	303
Total	**24**	40	**303**

Lampiran 3. Tahap Perkembangan Telur Karang *H. actiniformis* Bulan September 2006

Tahap Perkembangan Sperma	Bulan ¼	Bulan Purnama	Bulan ¾	Bulan Gelap
I	0	0	0	0
II	173	241	0	0
Total	**173**	**241**	**0**	**0**
III	65	294	0	0
IV	0	0	655	715
Total	**65**	**294**	**655**	**715**

Lampiran 4. Tahap Perkembangan Sperma Karang *H. actiniformis* Bulan Juli 2006.

Tahap Perkembangan Sperma	Bulan ¼	Bulan Purnama	Bulan ¾	Bulan Gelap
I	0	0	0	0
II	0	0	0	0
Total	**0**	**0**	**0**	**0**
III	25	45	2	4
IV	15	5	96	110
Total	**40**	**50**	**98**	**114**

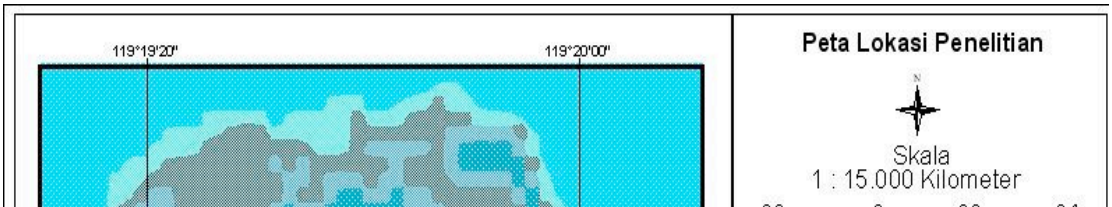
Lampiran 5. Tahap Perkembangan Sperma Karang *H. actiniformis* Bulan Agustus 2006.

Tahap Perkembangan Sperma	Bulan ¼	Bulan Purnama	Bulan ¾	Bulan Gelap (Tidak ada pengambilan sampel)
I	88	72	0	0
II	0	181	0	0
Total	**88**	**253**	**0**	**0**
III	0	0	59	0
IV	0	0	0	0
Total	**0**	**0**	**59**	**0**

Lampiran 6. Tahap Perkembangan Sperma Karang *H. actiniformis* Bulan September 2006.

Tahap Perkembangan Sperma	Bulan ¼	Bulan Purnama	Bulan ¾	Bulan Gelap
I	0	0	0	0
II	109	58	56	0
Total	**109**	**58**	**56**	**0**
III	84	0	0	68
IV	0	0	0	96
Total	**0**	**0**	**0**	**164**

Lampiran 7. Peta Lokasi Penelitian



ss

RIWAYAT HIDUP



Sri Asmi Romawanti, lahir di Selayar pada tanggal 03 Maret 1983, merupakan anak bungsu dari enam bersaudara, buah perkawinan Ayahanda Muh.Amin dengan Ibunda Syamsiah. Penulis memulai pendidikan formal pada tahun 1989 di SDN No.53 Cinimabela. Melanjutkan pendidikan Sekolah Lanjutan Tingkat Pertama di SLTPN 1 Benteng Selayar.

Appendix 3: Paper submitted by Leyla Knittweis to Conservation Genetics

1) Title

Genetic structure of *Heliofungia actiniformis* (Scleractinia: Fungiidae) populations in the Indo-Malay Archipelago: implications for live coral trade management efforts

2) Running Title

Population genetics of *Heliofungia actiniformis*

3) Names of Authors

Leyla Knittweis[*1], Wiebke Elsbeth Krämer[2], Janne Timm[2] and Marc Kochzius[2]

4) Institution Addresses

[1]Centre for Tropical Marine Ecology, Fahrenheitstrasse 6, 28359 Bremen, Germany

[2]Biotechnology and Molecular Genetics, FB2-UFT, University of Bremen, Leobenerstrasse, 28359 Bremen, Germany

5) Address of Corresponding Author

Leyla Knittweis
Centre for Tropical Marine Ecology, Fahrenheitstrasse 6, 28359 Bremen, Germany.
Tel: +49-421-2380058
Fax: +49-421-2380030
E-Mail: leyla.knittweis@zmt-bremen.de

6) Keywords

Mushroom coral; larval source; genetic variation; larval dispersal; Indo-Pacific.

Abstract

The fungiid *Heliofungia actiniformis* is one of the most popular scleractinian coral species in the growing live aquarium trade, with the majority of specimens originating in Indonesia. Details on population connectivity may potentially provide important information with regards to fishery management efforts. Phylogeographic structure was examined, using ribosomal ITS1, 5.8S and partial ITS2 sequences on a small scale among populations in the Spermonde Archipelago, South Sulawesi (up to 65 km distance), and on a large scale throughout the Indo-Malay Archipelago (up to 2,900 km distance). Significant genetic structuring was found at both scales. Within the Spermonde Archipelago isolation by

distance as well as local oceanographic features shaped patterns of genetic connectivity. On the large scale, the data revealed genetically distinct populations in Tomini Bay, New Guinea and the Thousand Islands near Jakarta, and a lack of genetic differentiation among populations lying close to or directly in the path of the Indonesian throughflow: from the central Visayas to the Flores Sea. Whilst the influence of both historical and present day processes on genetic structuring of *H. actiniformis* populations was revealed, large scale results further emphasised the importance of current dynamics on larval dispersal patterns in this species. Potential for larval input from surrounding populations, and the increased vulnerability of upstream as well as isolated populations should be taken into consideration when setting future harvest quotas.

Introduction

Marine ornamental species are collected around the world to supply specimens for the international aquarium trade, which is estimated to be worth 200-330 million US$ annually. Indonesia is the world's largest exporter of live marine ornamental corals since the 1980s (Wabnitz et al. 2003) and *Heliofungia actiniformis* ranks in the top five of all coral species being exploited for the live coral market (Green and Shirley 1999). An export quota system is in place, but knowledge on the population ecology of this species is limited (Abe 1937, 1940) and altogether lacking for the Indo-Malay Archipelago. Yet if harvesting regimes are to be sustainable in the long term, the exploited populations need to be self-sustaining, i.e. recruitment needs to exceed mortality and polyp collection. An indication of the extent of population connectivity provided by dispersing larvae is thus important with regards to the design of successful fishery management recommendations.

Due to the inherent difficulty in studying coral larval dispersal pathways in situ, most information to date has come from the study of the indirect effects of larval dispersal on the genetic differentiation and gene flow among populations (e.g. Ayre and Hughes 2000; Nishikawa et al. 2003; Magalon 2005; Vollmer and Palumbi 2007). However, a number of factors have been implicated in shaping present-day genetic structure of marine populations, including the roles of (1) larval dispersal capabilities (Ayre and Hughes 2000; Lourie et al. 2005), (2) present day physical oceanographic features (Williams and Benzie 1996; Rodriguez-Lanetty and Hoegh-Guldberg 2002), and (3) historical events (Nelson et al. 2000; Barber et al. 2002).

The dispersal potential of scleractinian coral species is influenced by the mode of sexual reproduction: in oviparous species this involves broadcast spawning of pelagic gametes, in viviparous species the release of brooded planulae. While brooded planulae generally settle within 1-2 days of release, planulae from spawning corals require 4-6 days after gamete release before they settle (see review by Harrison and Wallace 1990). The only existing study on the reproductive characteristics of *H. actiniformis* to date found that well developed, brooded larvae were released by the mother polyps. The larvae showed negative phototactic behaviour after 2 days and the great majority had attached to the substrates provided in the laboratory after 3 days (Abe 1937). In *H. actiniformis*, one may thus expect somewhat limited larval dispersal and potentially significant genetic structuring between neighbouring reef systems. However, it is known that coral larvae are able to prolong their competency periods if they do not encounter suitable substrate for settlement. Furthermore, although the larvae of brooding corals settle faster than those of broadcast spawners, their potential maximum competency periods are much longer due to the high energy reserves they can draw upon when needed (Richmond 1989; Wilson et al. 1998).

The oceanography of the Indo-Malay Archipelago is dominated by the Indonesian throughflow (ITF) current, which moves up to 19 million m^3 of water per second from the Pacific to the Indian Ocean (Gordan and Fine 1996; Gordon 2005). This current provides a potential dispersal corridor for marine larvae from the north-west Pacific along the Makassar Strait, all the way into the Flores and Banda Seas before finally being deflected to the Indian Ocean. Seasonally reversing east-west currents in the Java and Flores Seas moving at of up to 75 cm per second (Wyrtki, 1961) should further facilitate present-day marine larval dispersal within the Indonesian Seas.

However, the complex geographical history of the Indo-Malay Archipelago (Tomascik et al. 1997) has in the past provided numerous opportunities for vicariance. Between 2.5 million and 10,000 years ago, sea levels fell repeatedly during multiple glaciations (Haq et al. 1987). During the height of the Pleistocene glacial period about 18,000 years ago, sea levels are thought to have dropped by 130 m (Voris, 2000), greatly reducing the ITF and exposing both the Sunda and the Sahul continental shelf. Indeed, a number of studies have found the signatures of both the isolation of marine basins and postglacial re-colonisation events on the genetic structure of marine organisms in the Indo-Malay Archipelago (Nelson 2000; Barber et al. 2002; Sugama et al. 2002; Lourie et al. 2005).
Despite the central Indo-Pacific's ecological importance as the world's coral biodiversity hotspot (Hughes et al., 2002), and the subsistence value of local reef exploitation to millions of people, no previous investigations into patterns of genetic variation of scleractinian coral species in this region are known to the authors. We used ribosomal internal transcribed spacer (ITS) sequences to (1) examine genetic variability and genetic affinities of *H. actiniformis* populations on a small scale, i.e. throughout the Spermonde Archipelago (South Sulawesi) among reefs 7-65 km apart and (2) examine genetic variability and genetic affinities of *H. actiniformis* populations on a large scale, i.e. throughout the Indo-Malay Archipelago among reefs up to 2,900 km apart. We discuss the implications of likely larval connectivity patterns in light of potential management approaches to the exploitation of this species for the coral aquarium trade.

Materials and methods

Collection of samples

Heliofungia actiniformis tentacle clippings were collected from a total of ten sites throughout the Indo-Malay Archipelago (Fig. 1). Nine locations were in Indonesia: Adi, Gilli Trawangan, Komodo, Manado, Pulau Seribu, Saboeda, Pulau Sembilan, Spermonde Archipelago, Tilamuta and one in the Philippines: Cebu. In the Spermonde Archipelago samples were collected from nearshore (Barrang Lompo, Samalona), midshelf (Lanyukang, Sarappokeke) and outershelf (Kapoposang) reefs. Wherever possible, tissue samples were collected from polyps at least 50 m apart and of different colour morphs to reduce the likelihood of sampling genetically identical clones. Tissues were stored in 96% ethanol at 4 °C. Variable numbers of samples collected reflect species abundance levels at sampling sites.

Extraction, PCR amplification, and sequencing of DNA

DNA was extracted with a 5% Chelex® solution (Walsh et al. 1991) and approximately 650 base pairs of the ITS1-5.8S-ITS2 rDNA region were amplified by the polymerase chain reaction (PCR) using the coral specific primer A18S (GATCGAACGGTTTAGTGAGG; Takabayashi et al. 1998a) and universal primer ITS-4 (TCCTCCGCTTATTGATATGC; White et al. 1990). The PCR reaction was carried out in a total volume of 50µl. PCR reactions contained 1 µl template DNA, 10 mM TrisHCl (pH 9), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTP-mix, 0.4 mM of each primer, 40 µg BSA (bovine serum albumin) and 1 unit *Taq* polymerase (Promega). Amplification was performed in an Eppendorf Gradient S Thermal Cycler as follows: 30 cycles of 1 min at 94°C, 2 min at 55°C, 3 min at 72°C (Rodrigues-Lanetty and Hoegh-Guldberg, 2002). Successfully amplified PCR fragments were purified using the peqGOLD Cycle Pure Kit (Safety-Line; PeqLab Biotechnology GmbH, Erlangen, Germany) following the instructions of the manufacturer. Sequencing of both strands was conducted with the PCR primers, the DyeDeoxy Terminator chemistry (PE Biosystems, Foster City, U.S.A.), and an ABI automated sequencer.

Data analysis

The obtained sequences were aligned with a sequence of *Siderastrea stellata* (Forsman et al. 2005) using ClustalW (Thompson et al. 1994) as implemented in the software Bioedit

version 7.0.9 (Hall 1999) to delimit the ITS1, 5,.8S and ITS2 regions. The best fit model of nucleotide substitution was calculated by performing hierarchical likelihood ratio tests with Modeltest version 3.06 (Posada and Crandall 1998), and considered in subsequent analyses.

Inter- and intra-population genetic diversity indices, such as haplotype diversity (*h*) and nucleotide diversity (π) (Nei 1987), were calculated with the programme Arlequin version 3.11 (Excoffier et al. 2005). Population structure was further examined using Φ_{st}, an analogue of conventional F-statistics which considers both the number of haplotypes and the number of mutations in the sequences being investigated. The analysis of molecular variance (AMOVA) as implemented in Arlequin was used to determine the proportion of genetic variation within and between populations. A hierarchical AMOVA was subsequently carried out to look into potential population groupings according to geographic as well as oceanographic affinities.

Estimates of pairwise Φ_{st} were plotted against geographical distance and significance of the resulting correlation was determined by a Mantle test (Manly 1994) as well as reduced major axis regression, using Isolation by Distance (IBD) Web Service version 3.14 (Jensen et al. 2005; http://ibdws.sdsu.edu). Geographic distances consisted of the present-day shortest path by sea between any two populations, calculated in Google Earth version 4.0.13. Analyses had a nested design, with (1) small scale geographic variations investigated for sampling sites within the Spermonde Archipelago and (2) large scale geographic variations investigated for the Indo-Malay Archipelago.

A haplotype network based on pairwise sequence differences was created using the programme TCS version 1.21 (Clement et al. 2000). This method defines connections among haplotypes that have a cumulative probability of 95% of being true following the parsimony principle. Using these connections and the inferred missing intermediate stages, TCS plots a network. Gaps in the alignment resulting from base insertion/deletion were treated as a fifth nucleotide state.

Results

Sequence Analysis

In total, 21 different haplotypes were defined by 22 polymorphic sites in 173 obtained sequences. The nucleotide sequences were submitted to the European Molecular Biological Laboratory (EMBL); accession numbers are AM849550-AM849570. The best fit model of nucleotide substitution was Jukes and Cantor + I (JC+I), with estimated base frequencies and invariable sites (i) of 0.9839. Within the 623 bp alignment, the length of ITS-1 was 239 bp (complete); 5.8S and ITS-2 were 157 bp (complete) and 217 bp (partial) respectively. The full ITS-1 segment was more variable than the partial ITS-2 segment, with an average variation of 7.9% and 1.4%, respectively, whilst the 5.8S region was identical in all sequences.

Genetic Diversity Measures

Haplotype diversity (*h*) within populations ranged from 0.40 at Saboeda to 0.87 at Lanyukang in the Spermonde Archipelago. Average nucleotide diversity (π) ranged from 0.0009 at Tilamuta to 0.0048 at Adi. Levels of haplotype and nucleotide diversity were not always correlated, the most striking example being Adi in New Guinea, which had one of the lowest haplotype diversities ($h = 0.5$), yet the highest nucleotide diversity (Table 1).

Population Subdivision

Weak but nonetheless significant genetic structuring was found within the Spermonde Archipelago ($\Phi_{st} = 0.093$, $p < 0.02$; Table 2). The pairwise Φ_{st} values revealed the largest genetic subdivision to be present between the site closest to the shore (Samalona) and the

sites at the shelf-edge (Kapoposang, Lanyukang). A significant genetic structure was also found between the populations in Barrang Lompo and Kapoposang (Table 3).
AMOVA showed high levels of genetic structuring in the Indo-Malay Archipelago (Φ_{st} = 0.26, p <0.0001; Table 4), indicating restricted gene flow between the populations investigated. Grouping the populations into the five groups (1) Adi (2) Barrang Lompo, Cebu, Gilli Trawangan, Manado, Kapoposang, Komodo, Lanyukang, Samalona, Sarappokeke, Pulau Sembilan (3) Pulau Seribu (4) Saboeda (5) Tilamuta, explained the maximum of the variation among the populations (Φ_{ct} = 0.32, p = 0.007; Table 5).
Isolation by distance analyses (Fig. 2) revealed a significant increase in genetic diversity with increasing geographic distance on the small scale within the Spermonde Archipelago as well as across the Indo-Malay Archipelago.
Of the 21 haplotypes, 10 were singletons and of the remaining 11 haplotypes, 8 were shared among populations. One haplotype occurred in 34% of all samples and was found in 13 populations. The minimum-spanning network reveals two clades, separated by six mutational steps (Fig. 3). Clade one contained haplotypes from Adi, Cebu, Gilli Trawangan, Komodo, Manado, Pulau Seribu, Pulau Sembilan, and Spermonde. Haplotypes from clade two (63% of total) were present in all populations (Fig. 1).

Discussion

Small scale genetic differentiation: Spermonde Archipelago

The significant genetic structure within the Spermonde Archipelago points to limited larval exchange on a small scale: the average distance over which limited dispersal was noted in this study is 52 km. Similarly restricted gene flow in brooding coral species has been found in other studies. For instance *Seriatopora hystrix* exhibits high levels of genetic differentiation along a 90 km stretch of the Great Barrier Reef (Ayre and Dufty, 1994) and *Pocillopora damnicornis* shows significant genetic subdivision among seven sites surrounding Lord Howe Island on the East Coast of Australia separated by only 1-6.5 km (Miller and Ayre, 2004). Previous studies on the genetic structure of marine population genetics within Spermonde Archipelago found no restricted gene flow for the giant clam *Tridacna crocea* (Nuryanto and Kochzius 2006) and the stomatopod *Haptosquilla pulchella* (Barber et al. 2002) using mitochondrial COI sequences. However, both species have significantly longer planktonic larval phases: up to 14 days for *T. crocea* (Lucas, 1994) and 4-6 weeks for *H. pulchella* (Barber et al. 2002).
The significantly different pairwise Φ_{st} values for the sites with the largest geographic separation and the fact that the data follow the isolation by distance model indicate that geographic separation is an important factor for the degree of larval connectivity between the populations sampled in Spermonde (Fig. 2, A). However, a closer look at the data also points towards an influence of local current patterns on local larval exchange patterns. Although detailed information on the oceanography of the Spermonde Archipelago is lacking, it is clearly influenced by the ITF, which passes through the Strait of Makassar. Sites located at or near to the shelf edge are likely to be subjected to stronger currents than those in shallower water located near the coastline (for a detailed bathymetric map of the Spermonde Archipelago see de Klerk, 1983). This may explain the lack of genetic structure found between the outershelf and midshelf populations of Kapoposang, Lanyukang, and Sarappokeke, which have a maximal geographic separation of 36 km. In contrast, gene flow between Samalona (nearshore) and Lanyukang (midshelf), separated by 33 km, is limited. Furthermore, whereas a number of private haplotypes were found in the nearshore populations, none were present at the midshelf and outershelf sites. Such private haplotypes are most likely to develop in populations which have a limited exchange of larvae with surrounding populations (Barber et al. 2006), again supporting the notion of more isolated nearshore populations of *H. actiniformis* in the Spermonde Archipelago.

Large scale genetic differentiation: Indo-Malay Archipelago

At the large scale of the Indo-Malay Archipelago, long-distance gene flow between the Central Visayas and populations as far away as those in the Flores Sea was contrasted by regionally isolated populations in New Guinea, Tomini Bay, and the Java Sea. The presence of one large population with only limited genetic structure spanning the central part of the Indonesian Seas further supports the importance of current patterns in *H. actiniformis* larval dispersal: all populations lie very close to or directly in the path of the ITF current. A similar lack of genetic differentiation over a distance of 700 km was found for the coral *Pleisiastrea versipora* within the Ryuku Archipelago by Rodriguez-Lanetty and Hoegh-Guldberg (2002), who suggested dispersal aided by the strong Kuroshio Current as a likely explanation. Furthermore, within the Indo-Malay Archipelago, high levels of gene flow have previously been found along the path of the ITF: from North-Eastern Borneo to Southern Sulawesi for the prawn *Paneus monodon* (Sugama et al. 2002), from Northern Sulawesi and Northern Borneo to South Sulawesi for the boring giant clam *Tridacna crocea* (Nuryanto and Kochzius, 2006), from Northern Sulawesi to the Flores Sea for the stomatopods *Haptosquilla glyptocerus* and *Gonodactylinus viridis* (Barber et al. 2006), and from Cebu in the Philippines to West Timor for the pink anemonefish *Amphiprion perideraion* (Hamid 2007).

Considering the ITF reaches current strengths of up to 1m s^{-1} (Wyrtki 1961) and the potentially long competency periods of brooded coral larvae (Richmond 1989), it may be conceivable that *H. actiniformis* larvae are carried all the way from the Philippines to the distant reefs in the Flores Sea. However, it is more likely that dispersal is occurring via a series of stepping stones (Kimura and Weiss 1964). This notion is given support by the significant isolation by distance analysis at the large scale of the Indo-Malay Archipelago and the genetic break between the Flores Sea and New Guinea. Although the populations at Adi and Saboeda are potentially connected to those in the Flores Sea by an offshoot of the ITF (Fig. 1), potential stepping stones are lacking over considerable distances in this region. The limited number of samples collected in New Guinea combined with the curious presence of genetic differentiation between Adi and Saboeda, despite the islands' proximity and the presence of linking currents between the two sites (Gordon, 2005), does however suggest that the results should be treated with some caution.

H. actiniformis populations in Tomini Bay were genetically dissimilar to all other populations sampled in this study. This is a region with numerous endemic species (Wallace et al. 2003), and a similar pattern of restricted gene flow with surrounding populations was found in *H. pulchell*a by Barber et al. (2002). A likely explanation lies in the oceanographic properties of the area: there are no major currents driving an exchange of water masses with the neighbouring Malukku Sea (Gordon 2005). However, a genetic break between the neighbouring Java and Flores Sea *H. actiniformis* populations was also found, despite the presence of seasonally reversing currents in this region (Fig. 1). Indeed, a number of studies found similar genetic structuring between populations lying on the Sunda Shelf's Java Sea and those in the Flores Sea (Lourie et al. 2005; Barber et al. 2006; Nuryanto & Kochzius 2006; Timm & Kochzius, 2006; Hamid 2007). The most likely explanation is that these populations may still carry the genetic signature of re-colonisation events on the Sunda Shelf at the end of the last glacial period. As *H. actiniformis* is absent from the South China Sea but present on the western shores of Sumatra and Java (Veron 2000), re-colonisation may have come from populations in the Indian Ocean. This would agree with results from Timm and Kochzius (2006), who found high levels of gene flow between *Amphiprion ocellaris* populations on the Sunda Shelf and in the Indian Ocean. However, an extension of the current sampling regime into the Indian Ocean and at more sites on the Sunda Shelf would be needed to confirm this hypothesis.

The revealed pattern of two major clades (Fig. 3) nevertheless further supports this hypothesis. On the Sunda Shelf 93% of the haplotypes sampled belonged to clade 2. Also striking are the populations in Tomini Bay and at Saboeda (New Guinea), both of which only contained clade 1 haplotypes. Molecular genetic analyses of starfish (Williams and Benzie 1998; Benzie 1999; Kochzius et al. 2006), crustaceans (Duda and Palumbi 1999; Barber et al. 2002; Benzie et al. 2002) and fish (Bernardi et al. 2001, Timm & Kochzius 2006) all found a clear break between Indian and Pacific Ocean populations. In *H. actiniformis*, clade 1 haplotypes may have originated in the Pacific Ocean and the clade 2 haplotypes in the

Indian Ocean. Subsequent dispersal and mixing processes could have generated the modern day distribution patterns of the two clades. This would also agree with existing taxonomic appraisals of scleractinian corals in the region, which have indicated biogeographic patterns consistent with speciation events in the Indian and Pacific Oceans and ensuing dispersal into the central Indo-West Pacific (Wallace, 1997).
Furthermore, in terms of haplotype diversity, more diverse populations were found in these areas of presumed mixing, with Lanyukang (Spermonde) > Barrang Lompo (Spermonde) > Komodo, than on the periphery, where Saboeda < Adi < Pulau Seribu (Table 1). Patterns of nucleotide diversity somewhat mirrored these findings, with high values for Komodo > Manado > Sarapokeke (Samalona) / Pulau Sembilan where a frequent input of larvae from neighbouring populations is more likely than at the sites with lower values: Tilamuta < Pulau Seribu < Saboeda (Table 2). Benzie et al. (2002) likewise found diversity measures to be five times that of the average in the geographic centre of *Penaeus mondon* distribution and suggested the evolution of genetic variants in peripheral populations and their subsequent migration into the Indo-Malay Archipelago to be responsible for this pattern. Conspicuous in the present study is the population at Adi off western New Guinea. Here one of the lowest haplotype diversities (0.5) was contrasted by the highest nucleotide diversity (0.014) we found for all *H. actiniformis* populations investigated. It is feasible that haplotype variation is so limited due to the extreme rarity of mixing events at this location, but that the occasional input of larvae comes from three very different sources: the nearby Malukku Sea, the Banda Sea (via the ITF) and the Torres Strait. However, more detailed information on local oceanographic patterns and more samples from New Guinea are needed to draw firm conclusions.

Conclusions and management implications

The emerging pattern of *H. actiniformis* genetic population structure is thus highly complex, with the underlying pattern of isolation by distance as expected confounded by historical and contemporary oceanographic factors, especially at the scale of the Indo-Malay Archipelago (Fig. 2, B). The results do however for the first time give an indication of the present-day levels of population connectivity for *H. actiniformis* and presumably scleractinian coral species with similar ecological features in the Indo-Malay Archipelago, and can thus help to inform conservation and trade management decisions.
The apparent larval seeding of downstream ITF populations has several management implications. Firstly, harvesting at upstream populations should be limited and done with caution: over-harvested populations are likely to have slower recovery rates due to the more limited input of larvae into the system. Secondly, no-take areas at these locations are particularly important as they may serve as larval source regions. Although further research considering scleractinian coral species with a variety of reproductive modes is needed, the results of this study suggest existing reserves such as the chain of marine protected areas along the eastern and southern coast of Negros (Philippines), the Turtle Islands Park in northeast Borneo (Malaysia) and the National Park in Bunaken (Indonesia) (Spalding et al. 2002) should be strengthened, enlarged and ideally multiplied to support conservation efforts in the coral triangle.
Whilst it might be tempting to set less stringent quotas for those populations apparently lying downstream of potential source populations, restricted gene flow may occur within local reef systems, as evidenced by the pattern found within the Spermonde Archipelago in the present study, and also for coral populations within the Great Barrier Reef (Ayre et al. 1997, 2000). In the case of the Spermonde Archipelago, more stringent quotas should be set for nearshore reefs, where a larval input via the ITF is less likely than for mid- and outershelf reefs. More generally, the results underline the importance of considering the detailed characteristics of each harvesting area separately to achieve long term sustainability.
Finally, as for the upstream populations, isolated populations not connected by suitable stepping stones and/or sufficiently strong current regimes, should be treated with extreme prudence when harvesting quotas are set. Indeed, the government of Gorontalo province is currently considering whether to encourage fishermen to engage in the ornamental trade

(personal communications). Since the population in Tomini Bay is genetically isolated, collection of *H. actiniformis* and presumably a number of scleractinian coral species should be restricted and carefully monitored.

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References

Abe N (1937) Post-larval development of *Fungia actiniformis*. Palao Trop Biol Stn Stud 1:73-9

Abe N (1940) Growth of *Fungia actiniformis* var. palawensis Doederlein and its environmental conditions. Palao Trop Biol Stn Stud 2:105–145

Ayre DJ, Dufty S (1994) Evidence for restricted gene flow in the viviparous coral *Seriatopora hystrix* on Australia's Great Barrier Reef. Evolution 48(4): 1183-1201

Ayre DJ, Hughes TP, Standish RJ (1997) Genetic differentiation, reproductive mode, and gene flow in the brooding coral *Pocillipora damicornis* along the Great Barrier Reef, Australia. Mar Ecol Prog Ser 159:175-187

Ayre DJ, Hughes TP (2000) Genotypic diversity and gene flow in brooding and spawning corals along the Great Barrier Reef, Australia. Evolution 54: 1590-1605

Barber PH, Palumbi SR, Erdmann MV, Kasim Moosa M (2002) Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol Ecol 11:659-674

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. Evolution 60(9):1825-1839

Benzie JAH (1999) Genetic structure of coral reef organisms: ghosts of dispersal past. Am Zool 39:131-145

Benzie JAH, Ballment E, Forbes AT, Demetriades NT, Sugama K, Haryanti, Moria S (2002) Mitochondrial DNA variation in the Indo-Pacific populations of the giant tiger prawn *Panaeus monodon*. Mol Ecol 11:2553-2569

Bernardi G, Holbrook SJ, Schmitt RJ (2001) Gene flow at three spatial scales in a coral reef fish, the three spot dascyllus, *Dascyllus trimaculatus*. Mar Biol 138: 457-465

Clement M, Posada D, Crandall KA (2000) TCS: a programme to estimate gene genealogies. Mol Ecol 9:1657-1660

De Klerk LG (1983) Sea levels, reefs and coastal plains of Southwest Sulawesi, Indonesia; a morphogenetic-pedological study. Dissertation, State University of Utrecht

Duda TF Jr, Palumbi SR (1999) Population structure of the black tiger prawn *Panaeus monodon*, among western Indian Ocean and western Pacific populations. Mar Biol 134:705-710

Excoffier L, Laval G, Schneider S (2005) Arlequin version 3.0: an integrated software package for population genetics data analysis. Bioinformatics Online 1:47-50

Forsman ZH, Guzman HM, Chen CA, Fox GE, Wellington GM (2005) An ITS region of Sidastrea (Cnidaria: Anthozoa): is *S. glynni* endangered or introduced? Coral Reefs 24(2):342-347

Gordon AL, Fine RA (1996) Pathways of water between the Pacific and Indian Oceans in the Indonesian seas. Nature 379:146-149

Gordon AL (2005) Oceanography of the Indonesian Seas and their throughflow. Oceanogr 18(4):15-27
Green EP, Shirley F (1999) The global trade in corals. World Conservation Monitoring Centre, World Conservation Press, Cambridge, UK
Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95-98
Hamid LMO (2007) Genetic population structure of the pink anemonefish *Amphiprion perideraion* (Pomacentridae) in the Indo-Malay Archipelago. MSc Dissertation, University of Bremen
Harrison PL, Wallace CC (1990) Reproduction, dispersal and recruitment of scleractinian corals. In: Dubinsky Z (ed) Ecosystems of the World, vol. 25. Coral reefs Elsevier Science Publishers, Amsterdam, pp133-207
Haq BU, Hardenbol J, Vail PR (1987) Chronology of fluctuating sea levels since the Triassic. Science 235:1156-1167
Hughes TP, Bellwood DR, Connnolly SR (2002) Biodiversity hotspots, centres of endemicity, and the conservation of coral reefs. Ecol Lett 5:773-784
Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. BioMed Cent Genet 6:13
Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics 49:561-576
Kochzius M, Hauschild J, Kirchhoff S, Meyer-Wachsmuth I, Seidel C (2006) Congruence of genetic population structure in the blue starfish *Linckia laevigata* and its parasite *Thyca crystallina* in the central Indo-Malay Archipelago? In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany,19-22
Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: Hippocampus). Mol Ecol 14:1073-1094
Lucas JS (1994) The biology, exploitation and mariculture of giant clams (Tridacnidae). Rev Fish Sci 2:181-223
Magalon H, Adjeroud M, Veuille M (2005) Patterns of genetic variation do not correlate with geographical distance in the reef-building coral *Pocillopora meandrina* in the South Pacific. Mol Ecol 14:1861-1868
Manly BJF (1994) Multivariate statistical methods: a primer. Chapman and Hall, New York
Miller KJ, Ayre DJ (2004) The role of sexual and asexual reproduction in structuring high latitude populations of the reef coral *Pocillopora damicornis*. Heredity 92:557-568
Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000) Phylogeographic structure of the false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda Shelf. Mar Biol 137:727-736
Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York
Nishikawa A, Katoh M, Kazuhiko S (2003) Larval settlement and gene flow of broadcast-spawning (*Acropora tenuis*) and planula-brooding (*Stylophora pistillata*) corals. Mar Ecol Prog Ser 256:87-97
Nuryanto A, Kochzius M (2006) Limited gene flow in the giant clam *Tridacna crocea* across the Indo-Malay Archipelago. In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany, 19-22 September 2006
Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. Bioinformatics 14:817-818
Richmond RH (1989) Competency and dispersal of spawned versus brooded coral planula larvae. Paper presented at the 6th International Coral Reef Symposium, Townsville, Australia, 08-12 September 1988
Rodriguez-Lanetty M, Hoegh-Guldberg O (2002) The phylogeography and connectivity of the latitudinally widespread scleractinian coral *Pleisiastrea versipora* in the Western Pacific. Mol Ecol 11:1177-1189
Spalding MD, Ravilious C, Green EP (2001) World atlas of coral reefs. UNEP World Conservation Monitoring Centre. University of California Press, Berkeley, USA

Sugama K, Haryanti, Benzie JAH, Ballment E (2002) Genetic variation and population structure of the giant tiger prawn, *Penaeus monodon*, in Indonesia. Aquaculture 205:37-48

Takabayashi M, Carter D, Ward S, Hoegh-Guldberg O (1998) Inter- and intra-specific variability in ribosomal DNA sequence in the internal transcribed spacer region of corals. In: Greenwood JG, Hall NJ (eds) Proceedings of the Australian Coral Reef Society 75th Anniversary Conference, The University of Queensland, Brisbane, 1997

Thompson JG, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680

Timm J, Kochzius M (2006) Strong genetic structure and limited gene flow between populations of the False Clown Anemonefish (*Amphiprion ocellaris*) in the Indo-Malay Archipelago. In: Abstracts of the 6th European meeting of the International Society for Reef Studies (ISRS), Bremen, Germany, 19-22 September 2006

Tomascik T, Mah AJ, Nontji A, Moosa MK (1997) The Ecology of the Indonesian Seas Part One. The Ecology of Indonesia Series Volume VII. Periplus, Singapore

Veron JEN (2000) Corals of the world. AIMS Townsville, Australia

Vollmer SV, Palumbi SR (2007) Restricted gene flow in the Caribbean staghorn coral *Acropora cervicornis*: Implications for the recovery of endangered reefs. J Hered 98(1):40-50

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems, time durations. J Biogeogr 27:1153-1167

Wabnitz C, Taylor M, Green E and Razak T (2003) From ocean to aquarium. UNEP-WCMC, Cambridge, UK

Wallace CC (1997b) The Indo-Pacific centre of coral diversity re-examined at species level. Proceedings of the 8th International Coral Reef Symposium, Panama, 24-29 June, 1997

Wallace CC, Paulay G, Hoeksema BW, Bellwood DR, Hutchings PA, Barber PH, Erdmann M, Wolstenholme J (2003) Nature and origins of unique high diversity reef faunas in the Bay of Tomini, Central Sulawesi: The ultimate 'centre of diversity'? Proceedings of the 9th International Coral Reef Symposium, Bali, 23-29 October 2000

White TJ, Bruns T, Lee S, Taylor WJ (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications, Academic Press, San Diego, pp315-322

Walsh PS, Metzger DA, Higuchi R (1991) Chelex-100 as a medium for simple extraction of DNA for PCR based typing from forensic material. Biotechniques 10:506-513

Williams ST, Benzie JAH (1996) Genetic uniformity of widely separated populations on the coral reef starfish *Tinckia laevigata* from the East Indian and West Pacific Oceans, revealed by allozyme electrophoresis. Mar Biol 126:99-107

Williams ST, Benzie JAH (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by colour morphs, mtDNA, and allozyme data. Evolution 52(1):87-99

Wilson JR, Harrison PL (1998) Settlement-competency periods of larvae of three species of scleractinian corals. Mar Biol 131:339-345

Wyrtki K (1961) Physical oceanography of the Southeast Asian waters. Naga Report 2, Scripps Institution of Oceanography, p 195

Figure Legends

Fig. 1 A Sampling sites in the Indo-Malay Archipelago. Ad: Adi; Ce: Cebu; Gi: Gilli Trawangan; Ko: Komodo; Ma: Manado; PS: Pulau Seribu; Sb: Saboeda; Se: Pulau Sembilan; Sp: Spermonde; Ti: Tilamuta. Pie diagrams show frequencies of clades 1 and 2 (see Fig. 3; Spermonde data pooled). Arrows indicate dominant (solid) and seasonally changing (dashed) currents. Shaded areas represent shelf regions exposed during the Pleistocene (taken from Voris 2000). ITF: Indonesian Throughflow. **B** Sampling sites in the Spermonde Archipelago. BL: Barrang Lompo; Kp: Kapoposang; La: Lanyukang; Sm Samalona; Sr: Sarappokeke. Shaded areas represent present day reefs (taken from Landsat ETM+ satellite image, acquisition year 2002).

Fig. 2 Isolation-by-distance plots. **A** Spermonde Archipelago; $y=-0.1071+6.106^{-03}x$, $r=0.69$, $R^2=0.48$, $p=0.0427$. **B** Indo-Malay Archipelago; $y=-0.1021+3.016^{-04}x$, $r=0.45$, $R^2=0.2$, $p=0.0104$. Negative genetic distances were set to zero.

Fig. 3 Unrooted minimum-spanning network showing 21 haplotypes grouped into two clades. Square: ancestral haplotype (n=59). Size of ovals/square: proportional to haplotype frequencies. Lines represent single mutational steps, empty circles missing intermediate haplotypes.

Figures

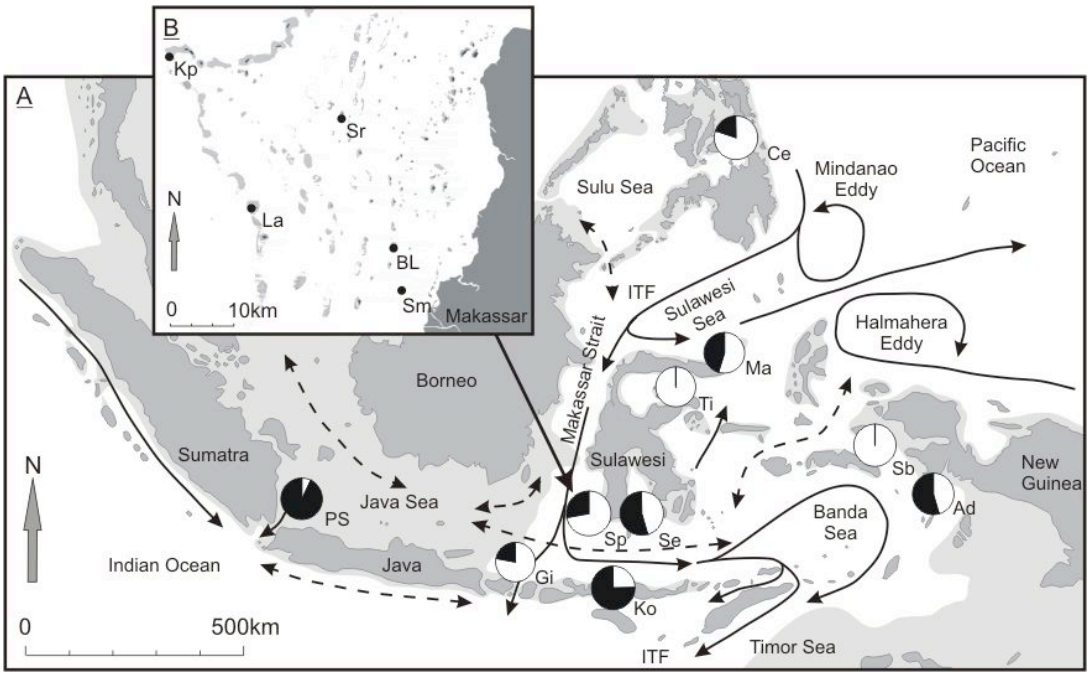


Fig. 1

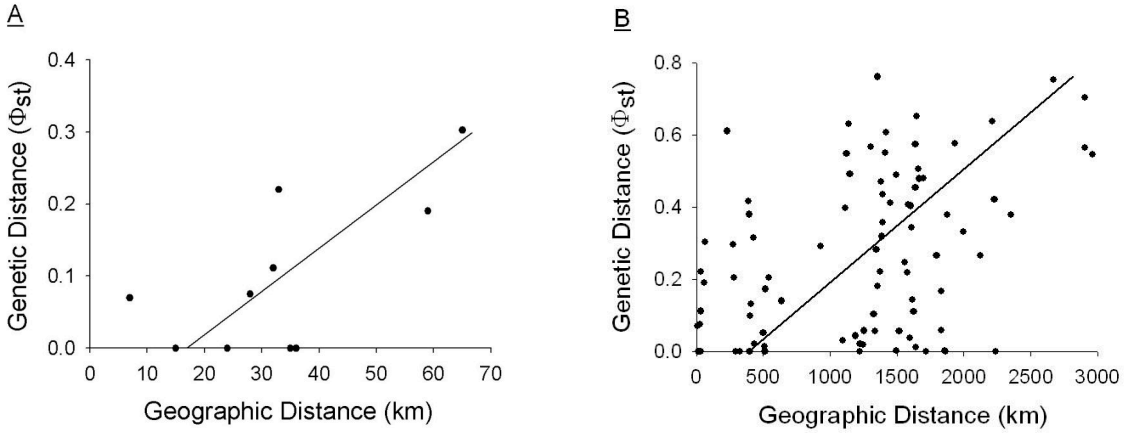

A
Genetic Distance (Φst)
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
Geographic Distance (km)
B
Genetic Distance (Φst)
0.8
0.6
0.4
0.2
0.0
0
500
1000
1500
2000
2500
3000
Geographic Distance (km)

Fig. 2

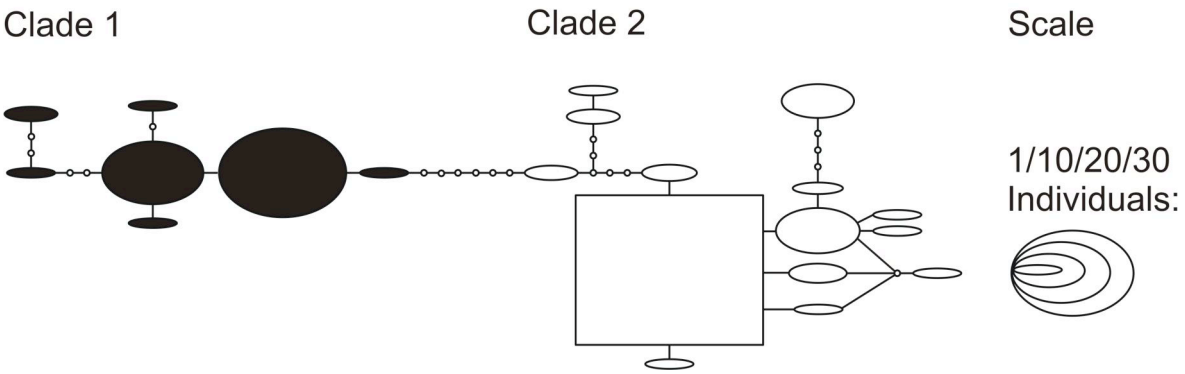


Fig. 3

Tables

Table 1 Sampling localities, sample size and genetic diversity measures ± standard deviation..

Sampling Site	Acronym	n	h	π
Adi	Ad	4	0.50 (±0.27)	0.0048 (±0.0038)
Barrang Lompo	BL	25	0.83 (±0.05)	0.0021 (±0.0015)
Cebu	Ce	30	0.68 (±0.08)	0.0025 (±0.0017)
Gilli Trawangan	Gi	9	0.69 (±0.15)	0.0025 (±0.0019)
Kapoposang	Kp	9	0.69 (±0.15)	0.0026 (±0.0019)
Komodo	Ko	8	0.82 (±0.10)	0.0036 (±0.0025)
Lanyukang	La	14	0.87 (±0.05)	0.0027 (±0.0019)
Manado	Ma	11	0.71 (±0.14)	0.0034 (±0.0023)
Pulau Seribu	PS	14	0.54 (± 0.11)	0.0015 (±0.0012)
Saboeda	Sb	5	0.40 (±0.24)	0.0020 (±0.0017)
Samalona	Sm	11	0.73 (±0.14)	0.0031 (±0.0022)
Sarapokeke	Sr	10	0.80 (±0.09)	0.0032 (±0.0022)
Pulau Sembilan	Se	11	0.78 (±0.12)	0.0032 (±0.0022)
Tilamuta	Ti	11	0.65 (±0.11)	0.0009 (±0.0009)

Table 2 Analysis of Molecular Variance (AMOVA) results: Spermonde Archipelago. Φ_{st} = 0.093, p = 0.022.

Source of Variation	Degrees of Freedom	Sum of squares	Variance component	% Variation
Among populations	4	7.680	0.08372	9.30
Within populations	64	52.262	0.81659	90.70
Total	68	59.943	0.90031	

Table 3 Matrix of pairwise population Φ_{st} values, Spermonde Archipelago. Sm: Samalona; BL: Barrang Lompo; Sr: Sarappokeke; La: Lanyukang; Kp: Kapoposang

	Sa	**Bl**	**Sr**	**La**	**Kp**
Sa	0.000				
Bl	0.070	0.000			
Sr	0.111	-0.006	0.000		
La	0.220**	0.075	0.062	0.000	
Kp	0.302**	0.190*	0.004	-0.042	0.000

* $p<0.05$, ** $p<0.01$

Table 4 Analysis of Molecular Variance (AMOVA) results: Indo-Malay Archipelago. Φ_{st} = 0.26, p = <0.0001

Source of Variation	Degrees of Freedom	Sum of squares	Variance component	% Variation
Among populations	13	53.895	0.27809	25.92
Within populations	159	126.379	0.79484	74.08
Total	172	180.274	1.07293	

Table 5 Hierarchical Analysis of Molecular Variance (AMOVA) groupings. Ad: Adi; BL: Barrang Lompo; Ce: Cebu; Gi: Gilli Trawangan; Ko: Komodo; Kp: Kapoposang; La: Lanyukang; Ma: Manado; PS: Pulau Seribu; Sb: Saboeda; Se: Pulau Sembilan; Sm: Samalona; Sr: Sarappokeke; Ti: Tilamuta

Grouped Populations	Φ_{ct}	***p***
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad) (PS) (Sb) (Ti)	0.31994	0.00712
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad) (PS) (Se) (Sb) (Ti)	0.28380	0.00974
(Ad, BL, Ce, Gi, Ko, Kp, Ma, PS, Sb, Se, Sm, Sr, Ti)	0.25919	0.00000
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb) (PS) (Ti)	0.23216	0.01870
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb) (PS) (Se) (Ti)	0.19836	0.03345
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Se) (PS) (Ti)	0.17017	0.03227
(BL, Ce, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb, Ti) (PS)	0.15449	0.04465
(BL, Gi, Ko, Kp, Ma, Se, Sm, Sr) (Ad, Sb) (Ce) (PS) (Ti)	0.14260	0.08523
(BL, Gi, Ko, Kp, Se, Sm, Sr) (Ad, Sb) (Ce, Ma) (PS) (Ti)	0.13662	0.06510
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Ti) (Se) (PS)	0.12492	0.07738
(BL, Ce, Gi, Ko, Kp, Ma, Sm, Sr) (Ad, Sb, Se, Ti) (PS)	0.11662	0.05212
(BL, Gi, Ko, Kp, PS, Se, Sm, Sr,) (Ad, Sb) (Ce, Ma) (Ti)	0.05764	0.06165

2. Wichtigste Positionen des zahlenmäßigen Nachweises

Die wichtigsten Position des separat eingereichten zahlenmäßigen Nachweises waren die Personalmittel.

Sie wurden zur Anfertigung von zwei Dissertationen eingesetzt. Die anfänglichen Verzögerungen durch die geschilderten Zollprobleme konnten durch kostenneutrale Umwidmungen aus Reise-, Verbrauchs- und Chartermitteln aufgefangen werden.

Ein detaillierter Verwendungsnachweis wurde separat eingereicht.

3. Notwendigkeit und Angemessenheit der geleisteten Arbeit

Die geleistete Arbeit war notwenig und angemessen.

4. Voraussichtlicher Nutzen und Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Es wurden keine Erfindungen/Schutzrechtsanmeldungen gemacht und solche sind auch in Zukunft nicht vorgesehen. Da es sich um ökologische Grundlagenforschung handelt, sind kurzfristig keine unmittelbaren wirtschaftlichen Erfolgsaussichten nach Projektende zu erwarten. Demgegenüber ist ein mittelbarer Nutzen und eine mittelbare Verwertung der Ergebnisse langfristig gegeben. Die Ergebnisse stärken die wissenschaftliche Kapazität der Partnerinstitutionen und fließen in lokale Entscheidungsprozesse zur Ausweisung von marinen Schutzgebieten und Ökotourismus ein.

5. Während der Durchführung des Vorhabens dem ZE bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen

Es sind keine Ergebnisse dritter Seite bekannt geworden, die für die Durchführung des Vorhabens relevant sind. Es wurden regelmäßig Recherchen in Literaturdatenbanken durchgeführt. Ausserdem wurden die jeweils neunen Ausgaben der Fachzeitschriften (z.B. Coral Reefs, Marine Ecology Progress Series, Marine Biology, Limnology & Oceanography) gesichtet.

6. Erfolgte oder geplante Veröffentlichungen

Die wissenschaftlichen Projektergebnisse werden in wissenschaftlichen Fachzeitschriften publiziert. Drei Manuskripte sind eingereicht, an mehreren wird z.Z. gearbeitet (s. Pkt. 1).

Zuwendungsempfänger:	Förderkennzeichen:
Universität Bremen Fachbereich 2 Biologie/Chemie Biotechnologie und Molekulare Genetik	03F0390B

Vorhabensbezeichnung:

TP3.1 (UNHAS-UFT)

Molekulare Genetik als Werkzeug für das Management von marine Zierganismen in Sulawesi (Indonesien)

Laufzeit des Vorhabens:

01.01.2004 bis 30.06.2007

Berichtszeitraum: 1.1.2004-30.6.2007 (Abschlußbericht)

I. Kurze Darstellung

1 Aufgabenstellung

Im Rahmen des Projektes sollte die genetische Populationsstruktur von Korallenrifforganismen untersucht werden, die eine wichtige Rolle im Aquarienhandel spielen. Dies Untersuchungen wurden mit dem Ziel durchgeführt, das Verbereitungspotenzial dieser Arten abzuschätzen umd diese Information für Schutzmaßnahmen verfügbar zu machen.

2. Voraussetzungen, unter denen das Vorhaben durchgeführt wurde

Das Vorhaben wurde in enger Kooperation mit deutschen (ZMZ, AWI, AWI-Sylt) sowie indonesischen Partner (UNHAS, Universität Purwokerto) durchgeführt.

3. Planung und Ablauf des Vorhabens

Probenahme

Im Rahmen des Projektes erfolgte eine umfangreiche Probenahme an insgesamt 22 Standorten in Indonesien. Zusätzliches Probenmaterial von sechs Vergleichsstandorten wurde im Rahmen anderer Aktivitäten gesammelt oder von Kollegen zur Verfügung gestellt (Abbildung 3-1).

Es wurden Gewebeproben von 12 Anemonenfisch-Arten (Tabelle 3-1), 6 Riesenmuschelarten (Tabelle 3-2) und 6 weiteren Wirbellosen (Tabelle 3-3) gesammelt, sowie die Pilzkoralle *Heliofungia actiniformes* (in Zusammenarbeit mit Frau Leyla Knittweis, Teilprojekt 1.1) beprobt. Die Entnahme der Gewebeproben erfolgte so, daß die Tiere nicht aus ihrem Habitat entfernt und

nicht getötet werden mußte. Die Arten wurden nach folgenden Kriterien ausgewählt: (1) Bedeutung im Aquarienhandel (Evaluierung durch Besuche bei vier Großhändlern und Aquariengeschäften in Makassar im Mai/Juni 2004 und Literaturrecherche), (2) Häufigkeit in den Korallenriffen von Spermonde, (3) einfache Probenhame, und (4) sichere taxonomische Bestimmung im Habitat.

Es war möglich, mehr Proben zu sammeln als ursprünglich geplant war, so daß nun zusätzliches Material zur Verfügung steht, das in der zweiten Phase von SPICE bearbeitet werden kann.

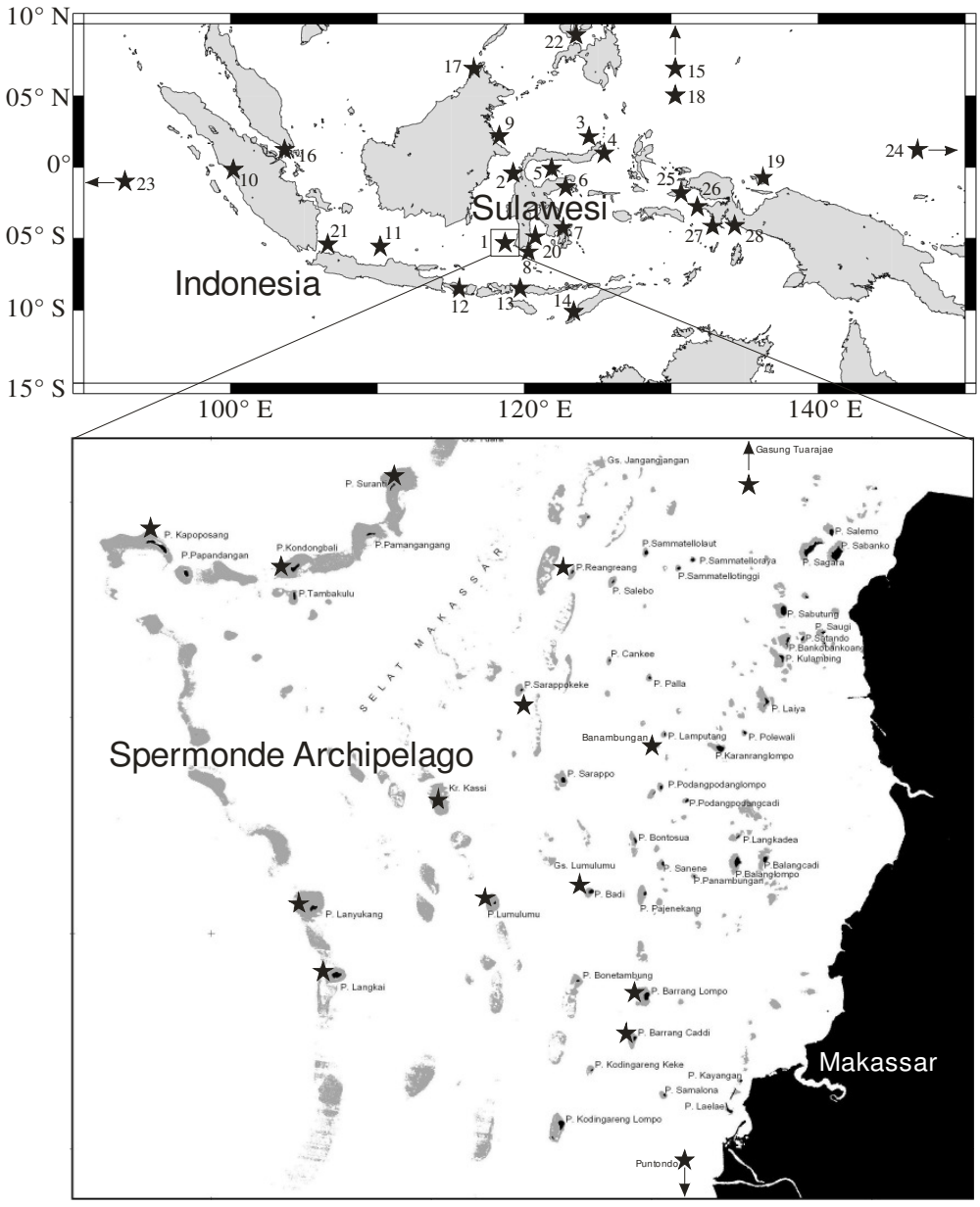


Abb. 3-1: Karte von Indonesien mit den Probenahmeorten (★): 1: Spermonde; 2: Donggala; 3: Manado; 4: Lembeh Strait; 5: Togian Islands; 6: Luwuk; 7: Kendari; 8: Bira (1-8: Sulawesi); 9: Sangalaki (Kalimatan); 10: Padang (Sumatra); 11: Karimunjawa (Java); 12: Padangbai (Bali); 13: Komodo; 14: Kupang (Timor); 15: Okinawa (Japan); 16: Batam (Riau); 17: Malaysia; 18: Taiwan; 19: Biak (Papua); 20: Pulau Sembilan (Sulawesi); 21: Pulau Seribu (Thousand Islands); 22: Cebu (Philippines); 23: Egypt; 24: French Polynesia; 25-28: Misol / Raja Ampat (Papua).

Tabelle 3-1: Proben von Anemonenfischen (Gattungen Amphirion and Premnas), die im Rahmen des Projektes gesammelt wurden.

	A. akallopisos	***A. clarkii***	***A. frenatus***	***A. melanopus***	***A. ocellaris***	***A. percula***	***A. perideraion***	***A. sandaracinos***	***P. biaculeatus***	***A. polymnus***	***A. akindynos***	***A. leucokranos***
Sulawesi/Spermonde	0	19	2	0	145	0	73	17	11	0	0	0
Sulawesi/Puntondo	0	0	0	0	11	0	0	0	0	0	0	0
Sulawesi/Donggala	0	23	0	0	23	0	36	3	6	0	0	0
Sulawesi/Manado	0	22	0	0	20	0	29	6	8	0	0	0
Sulawesi/Lembeh	0	8	0	1	0	0	9	7	2	0	0	0
Sulawesi/Togian Islands	0	19	0	0	25	0	0	2	8	0	0	0
Sulawesi/Luwuk	0	16	0	0	0	0	14	2	2	0	0	0
Sulawesi/Bira	0	23	0	0	17	0	21	10	4	0	0	0
Sulawesi/Kendari	0	10	0	0	37	0	31	11	2	0	0	0
Kalimantan/Sangalaki	0	4	0	0	37	0	21	9	0	0	0	0
Sumatra/Padang	27	15	0	0	17	0	0	0	0	0	0	0
Java/Karimunjawa	18	10	0	0	44	0	14	0	3	0	0	0
Bali/Pandangbai	0	20	0	1	14	0	7	0	0	0	0	0
Komodo/Komodo	0	17	0	1	14	0	10	2	3	0	0	0
Timor/Kupang	0	10	0	0	27	0	17	5	2	0	0	0
Aquarium shop (Makassar)	0	0	0	1	0	1	0	0	0	0	0	0
Cebu (Philippines)	o	20	0	0	20	0	20	0	10	1	0	0
Taiwan	0	9	1	0	0	0	2	0	0	0	0	0
Banggi-Islands (Malaysia)	0	14	0	0	24	0	12	4	2	4	0	0
Kota Kinabalu (Malaysia)	0	6	1	0	25	0	5	8	7	6	0	0
Batam (Riau)	0	0	6	1	23	0	0	0	0	0	0	0
Pulau Seribu (Java)	18	0	0	0	18	0	0	0	1	0	0	0
Biak (Papua)	0	19	0	2	0	13	25	2	1	0	2	1
Pulau Sembilan (Sulawesi)	0	6	0	0	8	0	0	0	5	0	0	0
Saropokeke (Spermonde)	0	7	0	0	0	0	1	7	0	0	0	0
Kapoposang (Spermonde)	0	15	0	0	5	0	8	0	1	0	0	0
Barranglompo (Spermonde)	0	0	0	0	3	0	1	0	1	0	0	0
Summe	**63**	**312**	**10**	**7**	**556**	**14**	**356**	**95**	**79**	**11**	**2**	**1**

Tabelle 3-2: Proben von Riesenmuscheln (Gattungen Tridacna and Hippopus), die im Rahmen des Projektes gesammelt wurden.

	T. crocea	*T. maxima*	*T. squamosa*	*T. gigas*	*T. derasa*	*H. hippopus*
Sulawesi/Spermonde	69	17	19	2	0	26
Sulawesi/Puntondo	0	0	0	0	0	0
Sulawesi/Donggala	1	2	4	0	0	0
Sulawesi/Manado	11	39	8	0	0	0
Sulawesi/Lembeh	1	2	3	0	0	0
Sulawesi/Togian Islands	98	53	6	0	0	0
Sulawesi/Luwuk	29	23	8	0	0	0
Sulawesi/Bira	13	13	13	0	0	0
Sulawesi/Kendari	29	4	13	0	0	0
Kalimantan/Sangalaki	27	9	13	1	0	1
Sumatra/Padang	8	26	6	0	0	0
Java/Karimunjawa	25	22	18	0	0	0
Bali/Pandangbai	1	6	6	0	0	0
Komodo/Komodo	30	23	18	0	1	0
Timor/Kupang	10	10	5	0	0	0
Malaysia	24	3	6	0	0	0
Batam (Riau)	0	0	4	0	0	0
Pulau Seribu (Java)	21	14	4	0	0	0
Biak (Papua)	36	25	3	0	0	0
Pulau Sembilan (Sulawesi)	30	14	6	0	0	0
Spermonde (Sulawesi)	9	20	10	0	0	0
Egypt	0	10	0	0	0	0
French Polynesia	0	20	0	0	0	0
Summe	**472**	**355**	**173**	**3**	**1**	**27**

Tabelle 3-3: Proben von Anemonen, Seesternen, Seescheiden und Schnecken, die im Rahmen des Projektes gesammelt wurden.

	Heteractis magnifica	***Entacmaea quadricolor***	***Heteractic crispa***	***Linckia laevigata***	***Polycarpa aurata***	***Thyca chrystallina***
Sulawesi/Spermonde	56	13	53	195	391	15
Sulawesi/Puntondo	6	2	0	0	10	0
Sulawesi/Donggala	5	9	33	43	35	26
Sulawesi/Manado	9	5	22	20	21	7
Sulawesi/Lembeh	2	3	10	32	40	11
Sulawesi/Togian Islands	5	6	6	32	46	11
Sulawesi/Luwuk	0	2	18	22	13	18
Sulawesi/Bira	9	6	22	20	17	18
Sulawesi/Kendari	15	13	35	27	31	18
Kalimantan/Sangalaki	22	1	3	32	31	47
Sumatra/Padang	45	2	0	0	0	0
Java/Karimunjawa	40	6	8	30	0	4
Bali/Pandangbai	9	3	11	0	0	32
Komodo/Komodo	5	27	28	24	27	22
Timor/Kupang	12	12	4	37	24	24
Cebu (Philippines)	0	10	10	20	20	0
Banggi-Islands (Malaysia)	12	5	1	95	11	30
Kota Kinabalu (Malaysia)	15	10	1	45	0	0
Batam (Riau)	19	22	0	0	0	0
Pulau Seribu (Java)	44	5	4	29	0	1
Biak (Papua)	8	7	0	30	24	5
Pulau Sembilan (Sulawesi)	0	6	4	28	16	26
Saropokeke (Spermonde)	0	1	0	1	0	0
Kapoposang (Spermonde)	5	8	15	8	11	12
Barranglompo (Spermonde)	1	2	4	0	0	0
Summe	**344**	**186**	**292**	**770**	**768**	**327**

Analyse der Proben

Im Rahmen des Projektes konnte die Populationsstruktur von 7 Fisch- und Wirbellosenarten indonesischer Korallenriffe bearbeitet werden, was die den Umfang der ursprünglichen Planung übertrifft. Das war hauptsächlich dadurch möglich, das 8 Projektstudenten und eine MSc-Studentin für das Projekt gewonnen werden konnten.

Alle untersuchten Arten zeigen eine signifikante Populationsstruktur, mit Ausnahme der auf dem blauen Seestern *Linckia laevigata* parasitirenden Schnecke *Thyca crystallina* (Tabelle 3-4). Detaillierte Forschungsergebnisse sind in den Abschnitten x-y dargestellt.

Tabelle 3-4: Φst-Werte für populationen von Korallenriff-Organismen im Indo-Malayischen Archipel. Signifikante Φst-Werte sind mit einem Sternchen markiert.

Art	**Φ_{st} - Wert**	***p*-Wert**	**Anzahl der Sequenzen**	**Länge der Sequenzen**	**mtDNA Marker**
Amphiprion ocellaris (Anemonenfisch)	0.21*	< 0.001	371	360 bp	Control region
A. perideraion (Anemonenfisch)	0.09*	< 0.001	248	400 bp	Control region
A. clarkia (Anemonenfisch)	0.02*	0.003	35	353 bp	Control region
Linckia laevigata (Blauer Seestern)	0.04*	< 0.004	257	520 bp	COI
Thyca crystalline (Parasitäre Schnecke)	0.02	0.1	181	409 bp	COI
Tridacna maxima (Riesenmuschel)	0.74*	< 0.001	211	484 bp	COI
Tridacna crocea (Riesenmuschel)	0.28*	< 0.001	300	456 bp	COI

Zusätzliche wurden noch die Pilzkoralle *Heliofungia actiniformis* (in Zusammenarbeit mit Frau Leyla Knittweis (siehe Teilprojekt 1.1 "marine protected areas and reef resources") und die Anemone *Heteractis magnifica* untersucht. Die populationsgenetische Studie an *H. magnifica* zeite, das der angewendete genetische Marker (Fragment des mitochondrialen COI-Gens) so gut wie keine Variation im Indo-Malayischen Archipel zeigt.

4. Wissenschaftlicher und technischer Stand, an dem angeknüpft wurde

Indonesien ist weltweit Hauptexporteur von marinen Aquarienfischen (US$ 5.5 Millionen im Jahr 1993, Wood 2001) und Korallen (US$ 2.0 Millionen im Jahr 1997, Green & Shirley 1999). Durch Überfischung sind manche Arten lokal ausgestorben oder zumindest nur noch sehr selten (DKP 2001, Wood 2001). Daher werden grundlegende Daten für ein Management und eine nachhaltige Nutzung die lebenden marinen Ressourcen dringend benötigt (Corbin 2001, Wood 2001).
Die Molekulargenetik ist ein wichtiges Werkzeug zur Untersuchung der Larvenverbereitung und des Gen-Flusses zwischen Populationen. Molekulare Marker für die Populationsgenetik sind z. B. mitochondriale DNA-Sequenzen (z.B. Kontrollregion) und Mikrosatelliten (Moritz 1995, Parker et al 1998, Sunnucks 2000), die aus Gewebeproben mittels der Polymerase-Kettenreaktion (PCR) gewonnen werden können (Mullis and Faloona 1987).
Untersuchungen der Populationsgenetik von marinen Organismen die im Aquarienhandel genutzt werden (Fische, Korallen und andere Wirbellose)stellen wichtige Informationen über ihren Status und für das Management zur Verfügung.
In diesem Forschungsprojekt sollen genetische Daten für die räumliche Anordnung von marinen Schutzgebieten erarbeitet werden. Marine Schutzgebiete sind eine Quelle für Larven sowie Rekruten, und können auch als sogenannte „stepping stones" für gestörte Küstenökosysteme, wie z.B. Korallenriffe, dienen (Roberts 1997).
Daten zur Konnektivität von Populationen sind wichtig, um Populationen zu identifizieren, die Larven in befischte Gebiete exportieren. Molekulare Studien a Wirbellosen der Korallenriffe haben gezeigt, das anhand vorherrschender Strömungsmuster die Verbreitung nicht gut abgeschätzt werden kann (Benzie 1999). Daher sind populationsgenetische Untersuchungen praktisch die einzige Möglichkeit die Verbreitungsmuster der Larven festzustellen. Informationen über Quellen und Senken sind eine Voraussetzung, um Managementstrategien für eine nachhaltige Nutzung zu entwickeln (Barber et al 2000). Obwohl diese Problem bekannt ist, wird es i.d.R. immer noch nicht für die räumliche Verteilung von marinen Schutzgebieten berücksichtigt.

Literatur

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) A marine Wallace's line? Nature 406:692-693

Benzie JAH (1999) Genetic structure of reef organisms: ghost dispersal of the past. American Zoologist 39:131-145

Corbin JS (2001) Marine Ornamentals '99, conference highlights and priority recommendations. Aquarium Science and Conservation 3:3-11

DKP (2001) Country status overview 2001 on reef fisheries exploitation and trade in Indonesia. Ministry of Marine Affairs and Fisheries (DKP), Telapak Indonesia Foundation, International Marinelife Alliance (IMA)

Greene E, Shirley F (1999) The global trade in coral. World Conservation Monitoring Centre. World Conservation Press, Cambridge

Moritz C (1995) Applications of mitochondrial DNA analysis in conservation: a critical review. Molecular Ecology 3:401-411

Mullis KB, Faloona FA (1987) Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. Methods in Enzymology 155:335-350

Parker PG, Snow AA, Schug MD, Booton GC, Fuerst PE (1998) What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79(2):361-382
Roberts CM (1997) Connectivity and management of Caribbean coral reefs. Science 278:1454-1457
Sunnucks P (2000) Efficient genetic markers for population biology. Trends in Ecology and Evolution 15(5): 199-203
Wood E (2001) Collection of coral reef fish for aquaria: global trade, conservation issues and management strategies. Marine Conservation Society, UK

5. Zusammenarbeit mit anderen Stellen

Die Arbeiten vor Ort in Indonesien fanden in Kooperation mit Dr. Jamaluddin Jompa und seinen Mitarbeitern von UNHAS, sowie Mr Agus Nuryanto von der Universität in Purwokerto statt.

Im Rahmen einer Kooperation mit Dr. Serge Planes (Universität Perpignan, Frankreich) konnte die Doktorandin Frau Janne Timm einen Monat zur Mikrosatelliten-Analyse im dortigen Labor arbeiten. Zudem gab es noch eine Zusammenarbeit mit dem Alfred-Wegener-Institut in Bremerhaven, so daß auch Mikrosatelliten-Analysen im dortigen Labor durchgeführt werden konnten

Dr. Marc Kochzius und der indonesischen Doktoranden Agus Nuryanto konnten vom 17.04. bis 06.05.2007 an einer GEO-Expedition nach Raja Ampat (Papua, Indonesien) teilnehmen. Während der Schiffsexpedition vom 21.04. bis 03.05.2007 konnten Proben in diesem sehr unzugänglichen Gebiet gesammelt werden. Die Expedition wurde von der Zeitschrift GEO finanziert, so daß für das Projekt keine Kosten entstanden sind.

II. Eingehende Darstellung

1 Verwendung der Zuwendung und erzielte Ergebnisse im Einzelnen

Wie bereits in der kurzen Darstellung beschrieben, wurde die Zuwendung für die populationsgenetische Untersuchung von 7 Fisch- und Wirbellosenarten indonesischer Korallenriffe genutzt. Die vorgegebenen Ziele wurden erreicht und die Forschungsergebnisse werden im folgenden detailliert dargestellt.

Strong genetic structure and limited gene flow between populations of the False Clown Anemonefish (*Amphiprion ocellaris*) in the Indo-Malay Archipelago

Janne Timm and Marc Kochzius

Manuscript in preparation for *Molecular Ecology*

Introduction

The False Clown Anemonefish (*Amphiprion ocellaris*) is distributed throughout the Indo-Malay Archipelago and it is one of the most popular aquarium fishes worldwide. It is caught from the wild in large numbers [8] but not much is known about its dispersal capabilities and the resulting connectivity between populations. The Indo-Malay Archipelago is located between the Indian and Pacific Ocean and consists of over 17,000 islands. The coral reefs around the islands are separated from each other by open water with complex current pattern [9]. Therefore, coral reefs do not provide a continuous habitat but rather a patchy environment. Additionally, the geographical history might have an influence on the population structures. Like many fishes on coral reefs, *A. ocellaris* has a life history with two different phases: adults are strongly site-attached, whereas larvae are planktonic [2]. Therefore, only the larvae have the potential to disperse, but the degree of dispersal potential depends on the period of the larval stage, which is only 8 to 12 days in *A. ocellaris*. Because of the dependence on anemones that occur in coral reefs and the short larval stage, the species has a high potential of population structuring. In this study, we investigated the genetic population structure and gene flow in *A. ocellaris* across the Indo-Malay Archipelago by analysing a fragment of the mitochondrial control region.

Material and Methods

378 tissue samples of *A. ocellaris* were taken by fin-clipping at different sites throughout the Indo-Malay Archipelago (Figure 1). The tissue was preserved in ethanol (96%) and DNA extraction was done with Qiagen and Macherey-Nagel Extraction Kits following the manufacturers' protocols.

A 370 bp long fragment of the mitochondrial control region was sequenced with the primers CR-A and CR-E [3] using the BigDye Terminator Cycle Sequencing Kit and an ABI Prism 310 automated sequencer according to the manufacturer's recommendations. Editing of the sequences was done with the program Seqman (DNASTAR) and a multiple alignment was done using ClustalW as implemented in the software BioEdit.

The phylogenetic Neighbor Joining analysis was performed with the software Paup* (ver. 4.0b10) 7 . The species *Amphiprion percula* (Pomacentridae) was used as outgroup. The computer programme ModelTest7 (ver. 3.06) [5] was used to determine the best-fit model of DNA evolution, which was used for the NJ analysis. An AMOVA analysis was performed and pairwise Fst values were calculated with Arlequin [6].

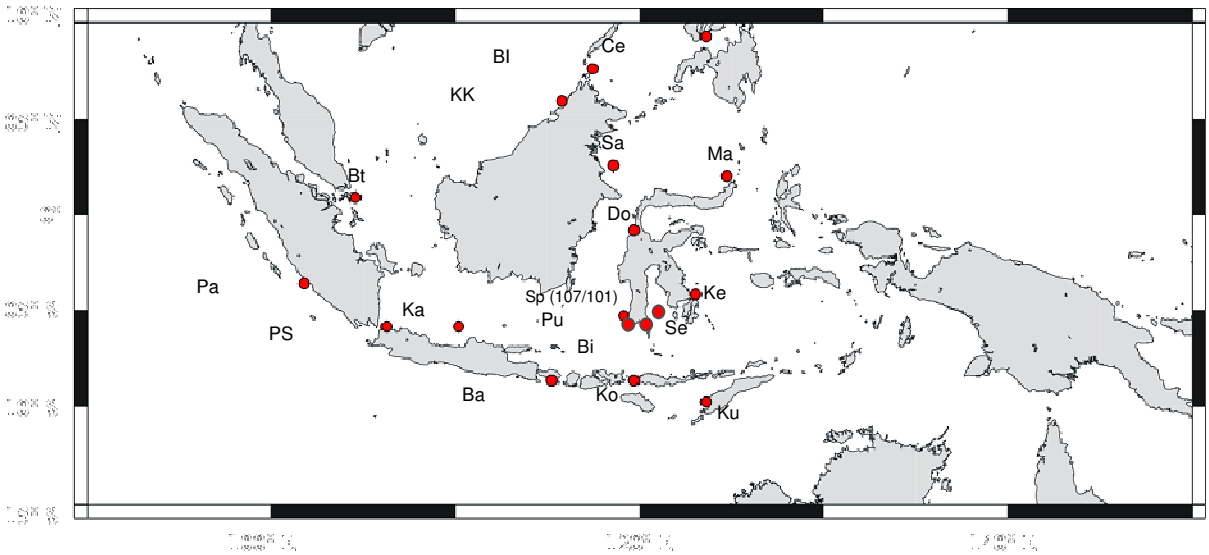


Figure 1: Sample sites in the Indo-Malay Archipelago. First number in brackets is the sample size and the second is the number of haplotypes. Abbreviations of locations: Pa – Padang, PS – Pulau Seribu, (Ka – Karimun Jawa, Ba – Bali, Ko – Komodo, Ku – Kupang, Sp – Spermonde, Pu – Puntondo, Bi – Bira, Se – Sembilan Islands, Ke – Kendari, Do – Donggala, Ma – Manado, Sa – Sangalaki, Ce – Cebu (Phillippines), BI – Banggi Islands (Malaysia), KK – Kota Kinabalu (Malaysia), Bt – Batam.

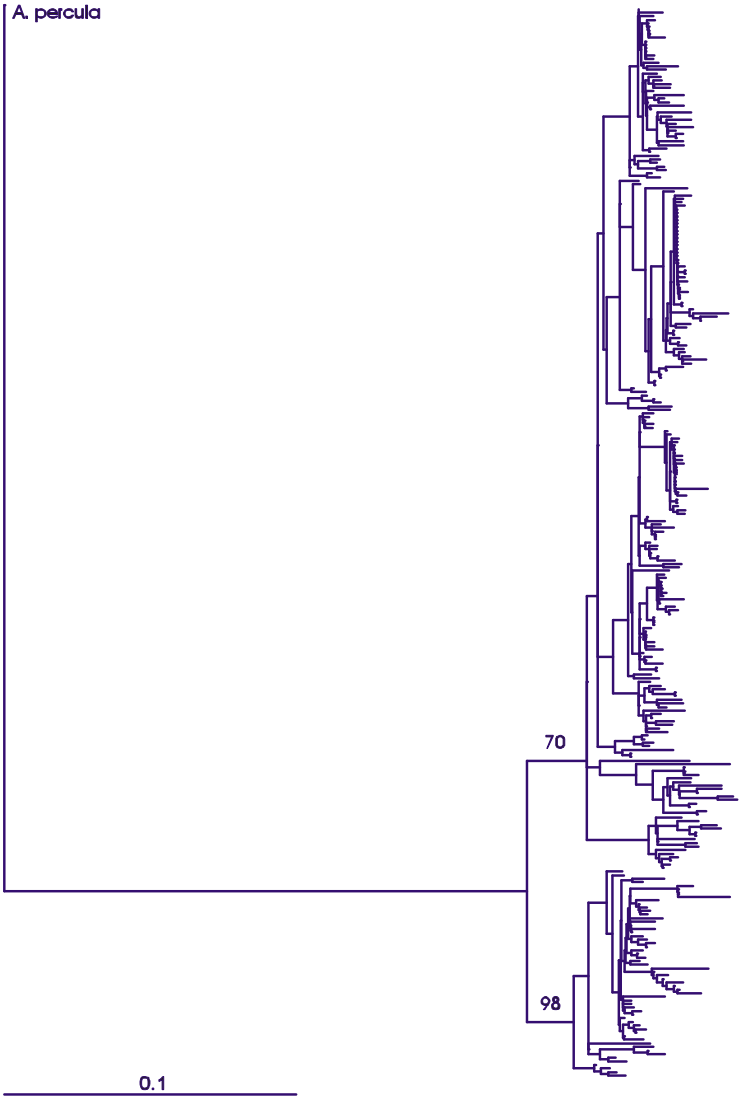


Figure 2: Neighbor-Joining Tree of *A. ocellaris* based on 370 bp of the mitochondrial control region. Given bootstrap values are obtained by 1000 Pseudoreplicates. *A.Percula* was used as outgroup. Clade2 includes all samples from Padang (Indian Ocean) and the majority of the samples from the Java Sea. All other sites are grouped in Clade1.

Results and Discussion

The phylogeographic analyses showed a clear separation of the populations from the Indian Ocean and Sunda shelf from all other populations indicated by distinct clades that are supported by high bootstrap values (Figure 2). This separation was also found in other organisms and could be explained by the separation of the Indian and Pacific Ocean during low sea-level stands[1] and re-colonisation of the Sunda shelf from the Indian Ocean. A population genetic analysis using AMOVA revealed a significant and

very high overall Φst-value of 0.2 ($p<0.001$), clearly showing limited gene flow between sampling sites. Pairwise comparisons show high connectivity only between the populations in the northern part of Borneo and Sulawesi, along the western coastline of Sulawesi and the Islands of Komodo and Kupang, which is facilitated by the strong currents of the Indonesian throughflow[4]. High pairwise F_{st} values from Padang (Indian Ocean) and the Java Sea populations support the strong differentiation of these populations already shown in the NJ-tree.

There is also a strong differentiation among Batam and the other populations, indicated by high pairwise F_{st} values. The population of Batam also shows a reduced genetic variability which could be the result of a late re-colonization after deglaciation or a recent bottleneck effect caused by extensive collection for the aquarium trade.

References

[1] Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) . A Marine Wallace Line? Nature; Vol. 406. **692-693.**

[2] Fautin DG & Allen GR (1994) . Anemonenfische und ihre Wirte. Tetra- Verlag, Melle.

[3] Lee WJ, Howell WH, Kocher TD (1995) . Structure and Evolutionof Teleost Mitochondrial Control Regions.J. Mol. Evol. **41**: 54- 66.

[4] Lukas R, Yamagata T, McCreary JP (1996). Pacific low-latitude western boundary currents and the Indonesian throughflow. Jounal of Geophysical Research; Vol. 101: 12,209-12,216.

[5] Posada D, Crandall K (1998) . Bioinformatics, 14: 817-818.

[6] Schneider S, Roessli D, Excoffier L (2000). **Arlequin** V. 2.000: A Software for Population Genetics Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

[7] Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4 Sinauer, Sunderland

[8] Wabnitz C, Taylor M, Green E, Razak T (2003). From Ocean to Aquarium. UNEP-WCMC, Cambridge, UK.

[9] Wyrtki K (1961) . Physical Oceanography of the Southeast Asian Waters. Naga Report Vol.2. University of Californis; Scripps Institute of Oceanography, La Jolla, California.

Evolution of anemonefishes (Amphiprioninae, Pomacentridae): Species boundaries in the Indo-Malay Archipelago

Janne Timm, Malgorzata Figiel, Marc Kochzius

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Abstract

Many species of coral reef fishes are distinguished by their colour patterns, but genetic studies have shown these are not always good predictors of genetic isolation and species boundaries. The genus *Amphiprion* comprises several species that have very similar colouration. Additionally, morphological characters are so variable, that sibling species can show a considerable overlap, making it difficult to differentiate them. In this study, we investigated the species boundaries between the sibling species pair *A. ocellaris* and *A. percula* and three closely related species of the subgenus *Phalerebus* (*A. akallopisos*, *A. perideraion*, *A. sandaracinos*) by phylogenetic analysis of mitochondrial control region sequences. Within the *A. ocellaris/A. percula* complex, five clades were found representing different geographic regions. Two major divergences with genetic distances of 19.6% and 20.4% indicate the presence of three deep evolutionary lineages in this group. The species of the subgenus *Phalerebus* show three monophyletic clades, concordant to the morphological classification.

Introduction

The Indo-Malay Archipelago, also called the "coral triangle", contains the world's richest marine biodiversity and is therefore a well-suited region to study divergence and speciation processes (Allen & Werner 2002). If the high diversity in this area is caused by an overlap of the Pacific and Indian Ocean faunas (Woodland 1983), or the result of an accumulation of species that evolved at the periphery (Jokiel & Martinelli 1992), or if it is actually the "center of origin" where species evolve (Briggs 2000, 2005) is discussed controversially.

In the genus *Amphiprion*, the latter theory was supported by Santini and Polacco (2006), who found the "center of origin" in an area reaching from the Philippines to the Great Barrier Reef and from Sumatra to Melanesia, which does not exclude speciation in peripheral remote areas (e.g. *A. tricinctus*, Marshall Islands endemic). Furthermore, a rather recent radiation was indicated in the Indian Ocean, because derived and endemic species are dominant (Santini & Polacco 2006).

In order to evaluate biodiversity correctly it is important to clarify species boundaries, integrities, and phylogenetic relationships (Frankham et al. 2002). Many species of coral reef fishes are distinguished by their colour patterns, but genetic studies have shown that these are not always sufficient indicators of genetic isolation and species boundaries (Bernardi et al. 2002). The genus *Amphiprion* comprises several species with very similar colouration. Additionally, morphological characters are so variable, that sibling species can show a considerable overlap, making it difficult to differentiate them.

There are closely related species that only show slight differences in their colour pattern in the subgenus *Phalerebus* (Allen 1991), such as *A. akallopisos* and *A. sandaracinos*. *A. akallopisos* has a white caudal fin and an orange to pinkish body colour, whereas *A. sandaracinos* has an orange caudal fin and its body colour is usually clearly orange. The white stripe on the back is supposed to be slightly longer in *A. sandaracinos,* spanning from the upper lip to the caudal peduncle, whereas in *A. akallopisos* it begins more on the forehead. However, this character seems to vary especially in the latter species. Regarding our observations, the white stripe often started also at the upper lip in *A. akallopisos*, so it is an overlapping and therefore rather weak character for distinguishing these two species. A more stable character separating the two species is the differently shaped teeth, indicating slightly different

ecological adaptations (Fautin & Allen 1994). *A. perideraion* has a similar body colour like *A. akallopisos*, but shows an additional white stripe between head and trunk. *A. perideraion* and *A. sandaracinos* show a sympatric distribution with the latter having a more restricted range and higher host specificity, accepting only two anemone species (*Heteractis crispa* and *Stichodactyla mertensii*) instead of four (*Heteractis crispa, H. magnifica, Stichodactyla gigantea*, and *Macrodactyla doreensis*) in *A. perideraion* (Fautin & Allen 1994). *A. akallopisos* has a parapatric distribution with the former two species, overlapping around the upper Sunda Islands and is also associated with only two anemone species (*Heteractis magnifica* and *Stichodactyla mertensii*; Fautin & Allen 1994; Figure 1).

The sibling species *A. ocellaris* and *A. percula* show more or less the same colour pattern, although *A. percula* is described to have larger black bands in its colouration (Fautin & Allen 1994), which could not be confirmed by our observations. There is rather a large variation: some specimens showing no black bands in their colouration while others do. Morphologically, these two species are differentiated by the number of spines in the dorsal fin, but also this character is overlapping: *A. ocellaris* has 10-11 and *A. percula* 9-10 spines. The ecological requirements of both species seem to be identical; both of them prefer the same host anemone species (*Heteractis magnifica* and *Stichodactyla gigantea*). Regarding Fautin & Allen (1994) these siblings have an allopatric distribution (Fig. 2), but Kuiter & Tonozuka (2004) reported both species in the Tomini Bay (Sulawesi), which indicates a parapatric distribution.

The high morphological similarity of the above mentioned species raises the question whether these species form distinct genetic clades.

This study aims to reveal (1) species boundaries within the anemonefish genus *Amphiprion* in the Indo-Malay Archipelago, and (2) speciation processes in the hotspot of marine biodiversity.

Materials and Methods

Sampling

A total of 73 tissue samples of six coral reef associated fish species of the genus *Amphiprion* were collected at different locations in the Indo-Malay Archipelago (see Tab.1 and Fig.3). The fishes were caught while underwater with two aquarium nets. A fin clip from the caudal fin was taken and the fishes were released into their host

anemones. It was therefore possible to obtain tissue samples without killing the animals. The samples were stored in 96% ethanol.

Fifteen samples of *A. ocellaris* were obtained from three different locations over a range of 1500 km. The sibling species *A. percula* was collected in Biak (New Guinea; 9 samples) and from New Britain (New Guinea; 4 samples). An additional number of 9 tissue samples have been collected from depths between 1 and 25 meters from the Togian Islands (Tomini Bay, Sulawesi). Since *A. ocellaris* is distributed across the Indo-Malay Archipelago (Fautin & Allen 1994), with its eastern border of distribution around the Molucca Islands and the most western tip of New Guinea, the samples from the Togian Islands have been classified as *A. ocellaris*.

Within the subgenus *Phalerebus*, 10 specimens from two locations in Indo-Malay Archipelago were analysed for each of the species *A. akallopisos*, *A. sandaracinos,* and *A. perideraion*.

Samples of *A. clarkii* were collected at the Indian Ocean coast of Sumatra (Padang) and in the Spermonde Archipelago (Sulawesi) (Table 1).

As outgroup for the phylogenetic analyses *Chromis viridis* was used, which is a member of the same family (Pomacentridae).

DNA extraction and amplification

Genomic DNA was extracted with filter column based extraction kits from Qiagen and Macherey-Nagel, following the manufacturers' protocols. A fragment with a maximum length of 420 bp of the mitochondrial control region was amplified by PCR with the primers CR-A (TTC CAC CTC TAA CTC CCA AAG CTA G) and CR-E (CCT GAA GTA GGA ACC AGA TG) (Lee et al. 1995). PCR was performed in a Perkin Elmer and Eppendorf Ep S Mastercycler with the following thermo-profile: 95°C for 2 minutes, followed by 35 cycles of 95°C for 30 seconds, 50°C for 30 seconds and 72°C for 60 seconds. The Terminal elongation was at 72°C for 2 minutes. 25 µl reactions contained 2.5 µl 10x PCR buffer, 0.075 µmol Mg^{2+}, 0.25 µmol dNTP mix, 10 pmol of each primer and 0.5 U Taq Polymerase. Between 10 and 30 ng genomic DNA was used of each sample as template. PCR products were purified with the QIA-quick PCR Purification Kit (Qiagen). Sequencing of both strands was conducted with the PCR primers using the Big Dye Terminator Cycle Sequencing Kit according to the manufacturer's recommendations and an ABI 310 and 3100 Automated Sequencer.

Phylogenetic analyses

Both strands were assembled and edited with help of the programme Seqman (Ver. 4.05, DNASTAR). Multiple sequence alignment was done using Clustal W (Thompson et al. 1994) as implemented in the software Bioedit (Ver. 7.0.0.1, Hall 1999).

The phylogenetic relationships of species were analysed by using Maximum parsimony (MP), Maximum likelihood (ML) and Neighbour joining (NJ) algorithms with the software Paup* (Ver. 4.0b10; Swofford 1998). The program Modeltest (Ver. 3.06; Posada 1998) was used to determine the best-fit model of DNA evolution for the present dataset. The statistical confidences were evaluated by 1000 non-parametric bootstrap replicates for NJ and MP analyses and by 100 for ML analysis. In order to test if the molecular clock can be used, the ML analysis was done with and without molecular clock enforced. The Shimodaira–Hasegawa test was used to verify if the topologies of the two ML trees are significantly different (Goldman et al., 2000; Shimodaira and Hasegawa, 1999).

Haplotype networks were drawn based on the results obtained from Arlequin (Schneider et al. 2000).

For all sequences the sequence divergences were calculated with Paup* (Swofford 1998) and the average for each population was taken for comparison.

Results

Phylogenetic tree

An alignment of 371 base pairs of the mitochondrial control region, containing 73 sequences from six species, was obtained. The alignment included several gaps. The best-fit model of evolution for the present dataset is the General Time Reversible model (GTR; Tavaré 1986) with a proportion of invariable sites of 0.15, and a gamma distribution shape parameter of 1.29, which was used for the NJ and ML analyses.

The phylogenetic analysis presented by a NJ phylogram (Figure 3) shows a grouping of all species in two main clades, both of them supported by high bootstrap values. One clade contains the species *A. sandaracinos*, *A. akallopisos*, and *A. perideraion*, as well as *A. clarkii*, the other *A. ocellaris* and *A. percula*.

The species of the subgenus *Phalerebus* (Allen 1991) form monophyletic sub-clades, well supported by bootstrap values between 98 % and 100 % for the MP and NJ

analysis. The ML analysis gave weaker bootstrap support for many branches. *A. sandaracinos* and *A. perideraion* form sister clades supported by bootstrap values of 90 % for NJ and 68 % for ML analysis and *A. akallopisos* is basal to them. A phylogeographic pattern is only visible for the species *A. perideraion*, where all but one sample of the population of Karimunjava from the Java Sea are grouped together with a strong bootstrap support. In *A. sandaracinos* two sub-clades are formed, both include sequences of the two studied locations, but in different proportions. *A. clarkii* is the sister clade to the species of the sub-genus *Phalerebus* (Figure 3).

The species *A. ocellaris* and *A. percula* form one well-supported monophyletic clade with a clear subdivision into an *A. ocellaris* and an *A. percula* sub-clade. The specimens from Togian Islands (Tomini Bay) are found within the *A. percula* clade. This clade shows a subdivision into three branches, corresponding to the three populations from Tomini Bay, Biak, and New Britain. The long braches and clear separations indicate a strong divergence between the populations.

A phylogeographic structure regarding the population from Padang (Indian Ocean) could be found in *A. ocellaris* (Figure 3).

Haplotype networks

The parsimonious haplotype network of the *A. akallopisos / perideraion / sandaracinos* complex (Figure 4) reveals three clearly separated clades. These clades are concordant to the morphologically defined species and no geographical pattern could be observed. The numbers of mutational steps in the *A. akallopisos* clade are six to 13, and in the *A. perideraion* clade one to 21. Two sub-clades, separated by 19 mutations, are present in *A. sandaracinos*. Within these sub-clades the variation was between one and four steps. The mutational steps between species pairs were 59 (*A. akallopisos - A. perideraion*), 53 (*A. perideraion - A. sandaracinos*), and 127 (*A. akallopisos - A. sandaracinos*).

The haplotype network of the *A. ocellaris / percula* species complex (Figure 5) shows that *A. ocellaris* is separated from *A. percula* collected in Tomini Bay by 80 substitutions. The genetic break between *A. ocellaris* individuals from Padang and its conspecifics from other sample sites is determined by 31 substitutions. This pattern is also obvious in the phylogenetic tree. Two to 25 mutational steps divide the other haplotypes within this species. The populations of *A. percula* in Tomini Bay and Biak

are divided by 43 steps. The population of *A. percula* from New Britain is strongly separated by 83 substitutions. Within each population of *A. percula* we find two to 26 mutational steps.

Sequence divergence

The genetic distances between the different species and populations are shown in Table 2. The distance of 12.1% between *A. percula* from Tomini Bay and Biak is between the maximum value within species (*A. ocellaris*; Padang – Spermonde = 9.7%) and the minimum value among species (*A. perideraion*; Karimunjava – *A. sandaracinos*; Kupang = 12.9%) observed in this dataset. The genetic distance between *A. percula* from Biak and New Britain is 19.6%; the distance between the population of Tomini Bay and New Britain is 20%. In comparison, the genetic differences between populations of *A. ocellaris* and *A. percula* are between 21% and 23%, but in the *A. akallopisos* / *sandaracinos* / *perideraion* complex they do not exceed 15.5% (Table 2).

Molecular clock

The Shimodaira-Hasegawa test, conducted to compare the ML trees reconstructed with and without the molecular clock enforced, showed no significant difference (p = 0.288). Therefore, the molecular clock could be applied and the separation among the species' ancestors could be estimated. The mutation rate of the mitochondrial control region in fish is approximately 3.6% per million years ± 0.46% (Donaldson & Wilson 1999). Therefore, a sequence divergence of 14.9% and 14.7% between *A. akallopisos* on the one hand and *A. perideraion* and *A. sandaracinos* on the other hand can be translated to about four million years. The separation between *A. perideraion* and *A. sandaracinos* was around 400,000 years later.

The sequence divergence between the sibling species *A. percula* and *A. ocellaris* is around 22%, which indicates that the split between them is five to six million years old. The divergence time between the Indian Ocean population of *A. ocellaris* and the other populations within the Indo-Malay Archipelago reaches back two to three million years. This is similar to the approximate separation of the populations of *A. percula* from Tomini Bay and Biak, which is around 3 million years old. The divergence

between Biak and New Britain is five to six million years old, similar to the split between *A. ocellaris* and *A. percula*.

Discussion

Phylogenetic analyses of closely related species provide insights into their genetic relationships, allowing us to verify their morphological taxonomic classification. Sometimes, such studies indicate that the previously assumed classification is wrong or not sufficient. This was the case in a study on the *Dascyllus trimaculatus* species complex (Bernardi et al. 2001), in which the authors revealed inconsistence between morphological, and colouration traits, and their molecular phylogenetic relationships. In contrast to that, research on coral reef fishes of the genus *Thalassoma* (Costagliola et al. 2004) confirmed the morphological species definition, even though the colouration pattern observed in that group of fishes questioned this. Additionally, such studies can provide insides into divergence and speciation processes, as well as geographic locations of these events.

Amphiprion akallopisos / perideraion / sanderacinos species complex

The definition by morphological characters and colouration pattern of *A. akallopisos, A. perideraion*, and *A. sandaracinos* can be supported by the molecular phylogenetic analysis in this study. Additionally, the short branches as well as the comparably small sequence divergences in this group confirmed the close relatedness of these species.

Based on the colouration pattern, it was expected that *A. akallopisos* and *A. sandaracinos* are sister species, but *A. sandaracinos* and *A. perideraion* were observed to be more closely related in the phylogenetic tree as well as in the haplotype network. This confirms the findings of another study on the molecular phylogeny of anemonefishes (Santini & Polacco 2006). The authors of this study assume that the main characters of the *A. akallopisos* / *perideraion* / *sandaracinos* species complex (orange-pinkish colouration with a white band on the back, a slender body with a rounded caudal fin) evolved before the radiation into different species.

Molecular clock estimates on the divergence time among the *Phalerebus* species indicate that the separation took place in the Pliocene, three to four million years ago. Due to climate oscillations in the Pliocene, the sea level dropped by around 40- 70 meters during glacial periods (Van Andel 1994). This caused intermediate frequent

disturbances that are likely to increase the probability of divergence and speciation (Roxburgh et al. 2003, Shea et al. 2004).

Since there was no major barrier in the Indo-Malay Archipelago during the inter-glacials of the Pliocene, the ancestor of the *A. akallopisos* / *sandaracinos* / *perideraion* complex probably inhabited both sides of the Sunda shelf. Sea level low stands during the glacials created a barrier for migration between the ocean basins. The population inhabiting the Indian Ocean gave rise to *A. akallopisos*, while the population on the Pacific side went through a sympatric speciation or allopatric speciation in separate ocean basins, such as the South China Sea, and the Java - Celebes Seas during sea level low stands (Voris 2000). This gave rise to *A. perideraion* and *A. sandaracinos*. The present distribution patterns of the species support these findings. *A. akallopisos* is distributed mainly in the Indian Ocean, but also present in the Java Sea and probably re-colonised the Sunda Shelf through the Sunda Strait after the glacial times. *A. sandaracinos* and *A. perideraion* both inhabit almost the whole Indo-Malay Archipelago. However, *A. sandaracinos* has a smaller distribution area (Fautin & Allen 1994). Although, there is an overlap in host anemone acceptance (both fish species can occur in *Heteractis crispa*), it seems to be that they avoid competition by specialisation on different host anemone species (Elliott & Mariscal 2001). *A. sandaracinos* represents a specialised and *A. perideraion* a rather generalised behaviour accepting two and four anemone species as hosts, respectively (Fautin & Allen 1994). The speciation of the latter two species took place around 400,000 years later than the split between *A. akallopisos* and the ancestor of *A. sandaracinos* and *A. perideraion.*

The separation of the Pacific and Indian Ocean by sea level low stands triggered divergence and speciation also in other coral reef dwelling animals (McMillan and Palumbi 1995, Williams 2000, Kochzius et al. 2003).

Amphiprion ocellaris / percula species complex

All specimens from the Togian Islands (Tomini Bay) are clearly included in the *A. percula* clade. This is contrary to the distribution pattern proposed by Allen (1991) and Fautin and Allen (1994), which describe the presence of *A. ocellaris* in Tomini Bay. Regarding Kuiter and Tonozuka (2005), both species occur in Tomini Bay, inhabiting different depth. *A. percula* is supposed to live in shallow water close to the

coastline, whereas *A. ocellaris* inhabits the deeper areas. We sampled at different locations and different depth varying between one and 25 meters, but could not confirm these findings, because the phylogenetic analysis clearly shows that all specimens belong to *A. percula*.

The specimens of *A. percula* collected at three different locations formed distinct geographic subclades with long branches and high bootstrap values. The haplotype network indicates a strong separation of the samples from New Britain (New Guinea), reaching back five to six million years. This is similar to the separation between *A. ocellaris* and *A. percula*. Nelson et al. (2000) found a similar divergence time between *A. ocellaris* and *A. percula* analysing fragments of the cytochrome *b* gene. Specimens of *A. percula* from Biak and from the Togian Islands are separated by a number of mutational steps similar to that one separating the population of *A. ocellaris* from Padang (Indian Ocean) to their conspecifics.

On the one hand, the genetic distances between some of the geographic groups of the *A. percula / ocellaris* complex are larger than between species in the genus *Phalerebus*. The clade of *A. percula* from New Britain shows a divergence of 83 steps from its conspecifics, raising the question if they can be still regarded as one species. On the other hand, the separation by 38 mutations between the *A. ocellaris* specimens from Padang (Indian Ocean) and the other *A. ocellaris* clade is lower than between the *Phalerebus* species. The same can be observed between *A. percula* samples from Tomini Bay and from Biak, although this value is comparable with the interspecific genetic distance of *A. perideraion* and *A. sandaracinos*.

The genetic distance of 12.1% between *A. percula* from Biak and *A. percula* from the Togian Islands could correspond to strongly diverged populations, whereas sequence divergences of 19.6% and 20.4% present between *A. percula* from New Britain (New Guinea) and the other *A. percula* populations are rather at species level. This view is supported by comparison with genetic distances among clearly distinct species within the genus *Amphiprion* (Table 2). The analysis reveals a sharp genetic break, although the strong coastal currents along the northern coast of New Guinea (Lukas et al. 1996) suggest high gene flow. Possibly, the clade of New Britain (New Guinea), separated from the other clades through the geographic distance of around 1000 km, is rather connected to populations of the Great Barrier Reef. These findings indicate a complex of five distinct lineages, three of them possibly representing closely related

species (*A. ocellaris*, *A. percula* from Tomini Bay and Biak, as well as *A. percula* from New Britain). As morphological characters are very similar, a verification of these findings by a nuclear molecular marker would be desirable. Additionally two lineages were detected which represent strongly diverged populations (from Padang in *A. ocellaris*, and from Biak in *A. percula*) in this complex. The basic differentiation pattern can be traced back to the separation of ocean basins during Pliocene sea level low stands (Van Andel 1994) During sea level low stands the Sunda Shelf was exposed and the population of *A. ocellaris* from Padang (Indian Ocean) was separated from the one of the other side of the Sunda shelf. Such a divergence of marine animals in the Indo-Malay Archipelago was also shown for other species, e.g. the mantis shrimp *Haptosquilla pulchella* (Barber et al. 2000, 2002), starfish species (Benzie 1999; Williams 2000) and the giant clam *Tridacna crocea* (Nuryanto & Kochzius, unpublished data). In butterflyfishes different species assemblages were found in the Pacific and Indic with genetic variations of around 2% between the species of the two different oceans. Comparably to the anemonefishes, butterflyfishes are also coral reef associated organisms, which seem to have undergone diversifications during sea level low stands in that area (Mcmillan & Palumbi 1995). Additionally, the central part of the Indo-Malay Archipelago was separated from the Pacific coast of New Guinea, leading to a genetic break between populations. Similar genetic differentiation was revealed in other marine organisms, such as *Haptosquilla pulchella* (Barber et al. 2002) and *Tridacna crocea* (Nuryanto & Kochzius, unpublished data).The population of *A. percula* from Tomini Bay is very isolated and obviously only connected to other populations of this species in the East by currents going through the Moluccan Islands. The population of mantis shrimps in Tomini Bay is also very divergent (Barber et al. 2002), supporting the distinctiveness of that area (Wallace et al. 2002). A study on scad mackerel found a connection between populations in Tomini Bay and the Moluccan Islands (Arnaud et al. 1999). It would be necessary to analyse populations of that area to proof this zone as a connection between Tomini Bay and the Pacific coast of New Guinea.

The results of the present study suggest that there are possibly three species present within the *A. ocellaris / percula* species complex, which is not concordant to the current taxonomy based on morphological characters and colour pattern. Within the *A. akallopisos / perideraion / sandaracinos* species complex, genetic clades follow species

boundaries defined by morphology and colour pattern. This study shows, that important factors for speciation of the genus *Amphiprion* in the „coral triangle" were Pliocene sea level fluctuations. This supports the "centre of origin" theory for explaining the high biodiversity present in that region (Briggs 2000, 2005).

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References

Allen GR (1991). Riffbarsche der Welt. Mergus Verlag, Melle, Germany, 1-272.

Allen GR & Werner TB (2002). Coral reef fish assessment in the "coral triangle" of southeastern Asia. Environmental Biology of Fishes 65, 209-214.

Arnaud S, Bonhomme F, Borsa P (1999). Mitochondrial DNA analysis of the genetic relationships among populations of scad mackerel (*Decapterus macarellus, D. macrosoma, D. russelli*) in South-East Asia. Mar. Biol. 135, 699-707

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000). A marine Wallace's line? Nature 406, 692-693.

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2002). Sharp genetic breaks among populations of *Haptosqulla pulchella* (Stomatopoda) indicate limits to larval transport patterns, causes and consequences. Mol. Ecol. 11, 659-674.

Benzie JAH (1999). Genetic structure of coral reef organisms: ghost of dispersal past. Amer.Zool. 39, 131-145.

Benzie JAH, Ballment E, Forbes AT, Demetriades NT, Sugama K, Moria HS (2002). Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon.* Mol. Ecol. 11, 2553–2569.

Bernardi G, Holbrook SJ, Schmitt RJ (2001). Gene flow at three spatial scales in a coral reef fish, the three- spot dascyllus, *Dascyllus trimaculatus*. Mar. Biol. 138, 457-465.

Bernardi G, Holbrook SJ, Schmitt RJ, Crane NL, DeMartini E (2002). Species boundaries, populations and colour morphs in the coral reef three-spot damselfish (*Dascyllus trimaculatus*) species complex. Proc. R. Soc. Lond. B 269, 599-605.

Briggs JC (2000). Centrifugal speciation and centres of origin. J. Biogeogr. 27, 1183-1188.

Briggs JC (2005). The marine East Indies: diversity and speciation. J. Biogeogr. 32, 1517-1522.

Costagliola D, Robertson DR, Guidetti P, Stefanni S, Wirtz P, Heiser JB, Bernardi G (2004). Evolution of the coral reef fish *Thalassoma* spp. (Labridae). 2. Evolution of the eastern Atlantic species. Mar. Biol. 144, 377-383.

Donaldson KA & Wilson RR (1999). Amphi-panamic geminates of snook (Percoidei: Centropomidae) provide a calibration of the divergence rate in the mitochondrial DNA control region of fishes. Mol. Phylogenet. Evol. 13, 208-213.

Elliott JK & Mariscal RN (2001). Coexistence of nine anemonefish species: differential host and habitat utilization, size and recruitment. Mar. Biol. 138, 23-36.

Fautin DG & Allen GR (1994). Anemonenfische und ihre Wirte. Tetra-Verlag, Melle, 1-168.

Frankham R, Ballou JD, Briscoe DA (2002). Introduction to Conservation Genetics. Cambridge Univ. Press, UK, 1-617.

Goldman N, Anderson JP, Rodrigo AG (2000). Likelihood-based tests of topologies in phylogenetics. Syst. Biol. 49, 652-670.

Hall TA (1999). BIOEDIT: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Series 41, 95-98.

Jokiel P & Martinelli FJ (1992). The vortex model of coral reef biogeography. J. Biogeogr. 19, 449-458.

Kochzius M, Soller R, Khalaf MA, Blohm D (2003). Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroidae) based on mitochondrial DNA sequences. Mol. Phylogenet. Evol. 28, 396-403.

Kuiter HK & Tonozuka T (2004). Indonesian reef fishes. Part 2. PT Dive and Dives, Bali, Indonesia, 376-377.

Lee WJ, Howell WH, Kocher TD (1995). Structure and evolution of teleost mitochondrial control regions. J. Mol. Evol. 41, 54-66.

Lukas R, Yamagata T, McCreary JP (1996). Pacific low- latitude western boundary currents and the Indonesian throughflow. J. Geogr. Res. 101, 12209-12216.

McMillan WO, Palumbi SR (1995). Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proc. R. Soc. Lond. 260, 229-236.

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000). Phylogeographic structure of false clownfish, Amphiprion ocellaris, explained by sea level changes on the Sunda shelf. Mar. Biol. 137, 727-736.

Posada D & Cranall K (1998). MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817-818.

Roxburgh SH, Shea K, Wilson JB (2004). The intermediate disturbance hypothesis: patch dynamics and mechanisms of species coexistence. Ecology 85, 359-371.

Santini S & Polacco G (2006). Finding Nemo: Molecular phylogeny and evolution of the unususal life style of anemonefish. Gene 385, 19-27.

Schneider S, Roessli D, Excoffier L (2000). ARLEQUIN version 2.000: A software program for population genetic data analysis. Genetic and Biometry Laboratory, Department of anthropology, University of Geneva, Switzerland.

Shea K, Roxburgh SH, Rauschert ESJ (2004). Moving from pattern to process: coexistence mechanisms under intermediate disturbance regimes. Ecol. Lett. 7, 491-508.

Shimodaira H & Hasegawa M (1999): Multiple Comparisons of Log- Likelihoods with applications to Phylogenetic Inference. Mol. Biol. Evol. 16, 1114-1116.

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (*and other methods). Version 4 Sinauer, Sunderland.

Tavaré S (1986). Some probabilistic and statistical problems on the analysis of DNA sequences. Lectures on Mathematics in the Live Sci. 17, 57-86.

Thompson JG, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Van Andel TH (1994). New views on an old planet: a history of global change. Cambridge Univ. Press, UK, 1-439.

Voris HK (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and durations. J. Biogeogr. 27, 1153- 1167.

Wallace CC, Paulay G, Hoeksema BW (2002). Nature and origins of unique high diversity reef faunas in the Bay of Tomini, Central Sulawesi: The ultimate "centre of diversity"? Proc. International Coral Reef Symp., Bali, Indonesia, 185-192.

Williams ST (2000) Species boundaries in the starfish genus *Linckia*. Mar. Biol. 136, 137-148.

Woodland DJ (1983). Zoogeography of the Siganidae (Pisces): an interpretation of distribution and richness patterns. Bull. Mar. Sci., 33, 713-717.

Figure 1: Distribution patterns and sample sites of the species *Amphiprion akallopisos* (dark grey, circles), *A. perideraion* (grey, stars) and *A. sandaracinos* (light grey, diamonds) in the Indo-Malay Archipelago (Fautin & Allen 1994).

Figure 2: Distribution patterns and sample sites of the species *Amphiprion ocellaris* (grey, stars) and *A. percula* (dark grey, circles) in the Indo-Malay Archipelago (Fautin & Allen 1994).

Figure 3: Neighbour-Joining tree of control region sequences (371 base pairs) of clownfish species from genus *Amphiprion* in the Indo- Malay Archipelago. Bootstrap values above branches are based on 1000 replicates for the NJ and MP (**bold**) analyses and below branches on 100 replicates for the ML (*italics*) analysis.

Figure 4: Haplotype network of *Amphiprion akallopisos, A. perideraion* and *A. sandaracinos* from different locations in the Indo-Malay Archipelago (grey shadings correspond to the locations, see legend). Hatches and numbers indicate the number of mutational steps.

Figure 5: Haplotype network of *Amphiprion ocellaris* and *A.* from different locations in the Indo-Malay Archipelago (for abbreviations see Table 1). Hatches and numbers indicate the number of mutational steps.

Table 1: Number of sampled individuals from 6 species of the genus *Amphiprion,* abbreviations used, accession numbers (EMBL), and the corresponding sample locations in the Indo-Malay Archipelago.

Species	Location	Abbreviation	Number of individuals	Number of haplotypes	Accession numbers
A. ocellaris	Spermonde, Sulawesi	AoSp	5	5	**AM747125 – AM747129**
	Banggi Islands, Sabah	AoBI	5	5	**AM747144 – AM747148**
	Padang, Sumatra	AoPa	5	5	**AM747139 – AM747143**
A. percula	Tomini Bay, Sulawesi	AperTI	9	9	**AM747130 – AM747138**
	Biak, New Guinea	AperBk	9	9	**AM747149 – AM747157**
	New Britain, New Guinea	AperNB	4	3	**AM745732 – AM745734**
A. akallopisos	Padang, Sumatra	AaPa	5	5	**AM747158 – AM747162**
	Karimun Jawa, Jawa Sea	AaKa	5	5	**AM747163 – AM747167**
A. perideraion	Manado, Sulawesi	ApMa	5	4	**AM747168 – AM747171**
	Karimun Jawa, Jawa Sea	ApKa	5	4	**AM747172 – AM747174**
A. sandaracinos	Manado, Sulawesi	AsMa	5	5	**AM747175 – AM747178**
	Kupang, Timor	AsKu	5	5	**AM747179 – AM747183**
A. clarkii	Spermonde, Sulawesi	AcSp	3	3	**AM747184 – AM747186**
	Padang, Sumatra	AcPa	3	3	**AM747187 – AM747189**

Table 2: Sequence divergences (calculated with Paup* considering the General Time Reversable model of evolution) between and within species of the genus *Amphiprion* from different locations in the Indo-Malay Archipelago (for abbreviations see Table 1).

	AoSp	AoBI	AoPa	AperTI	AperBk	AperNB	AaPa	AaKa	ApMa	ApKa	AsMa	AsKu	AcSp	AcPa
AoSp	0.065													
AoBI	0.052	0.039												
AoPa	0.097	0.096	0.038											
AperTI	0.230	0.225	0.220	0.063										
AperBk	0.236	0.227	0.221	0.121	0.043									
AperNB	0.231	0.219	0.210	0.204	0.196	0.012								
AaPa	0.246	0.235	0.242	0.288	0.278	0.290	0.036							
AaKa	0.247	0.235	0.241	0.289	0.276	0.290	0.029	0.026						
ApMa	0.259	0.253	0.258	0.296	0.281	0.312	0.155	0.151	0.049					
ApKa	0.251	0.245	0.246	0.296	0.286	0.309	0.144	0.138	0.049	0.018				
AsMa	0.251	0.239	0.249	0.271	0.264	0.286	0.152	0.150	0.138	0.132	0.027			
AsKu	0.254	0.242	0.252	0.267	0.259	0.282	0.148	0.145	0.133	0.129	0.024	0.018		
AcSp	0.258	0.255	0.254	0.279	0.272	0.289	0.172	0.170	0.188	0.183	0.201	0.196	0.020	
AcPa	0.242	0.237	0.239	0.271	0.258	0.270	0.167	0.165	0.192	0.179	0.193	0.188	0.058	0.013

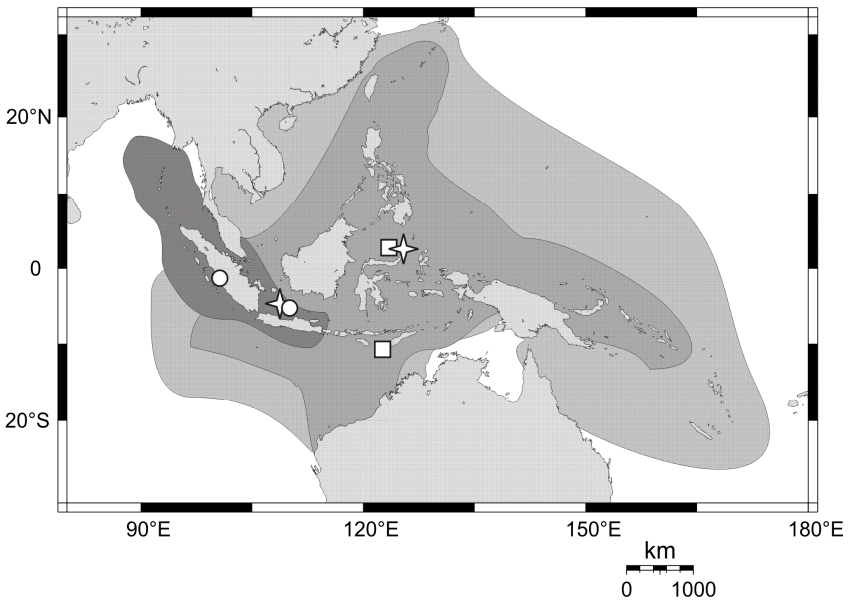


Figure 1

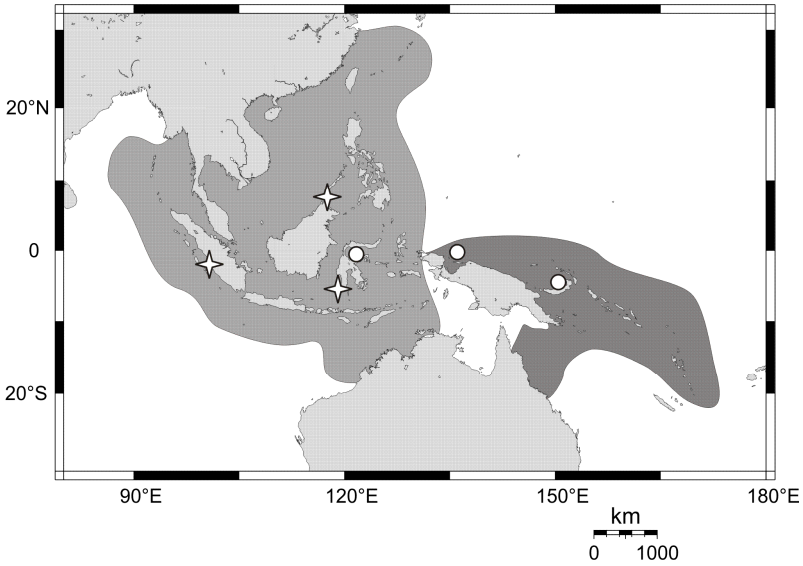


Figure 2

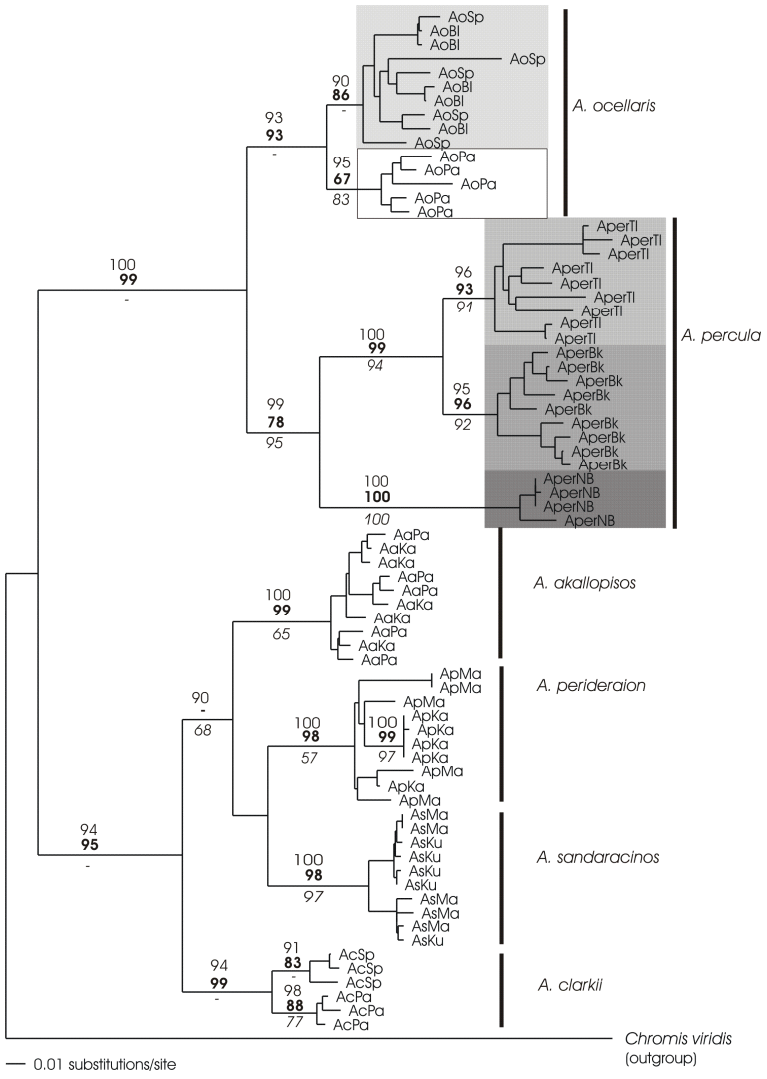


Figure 3

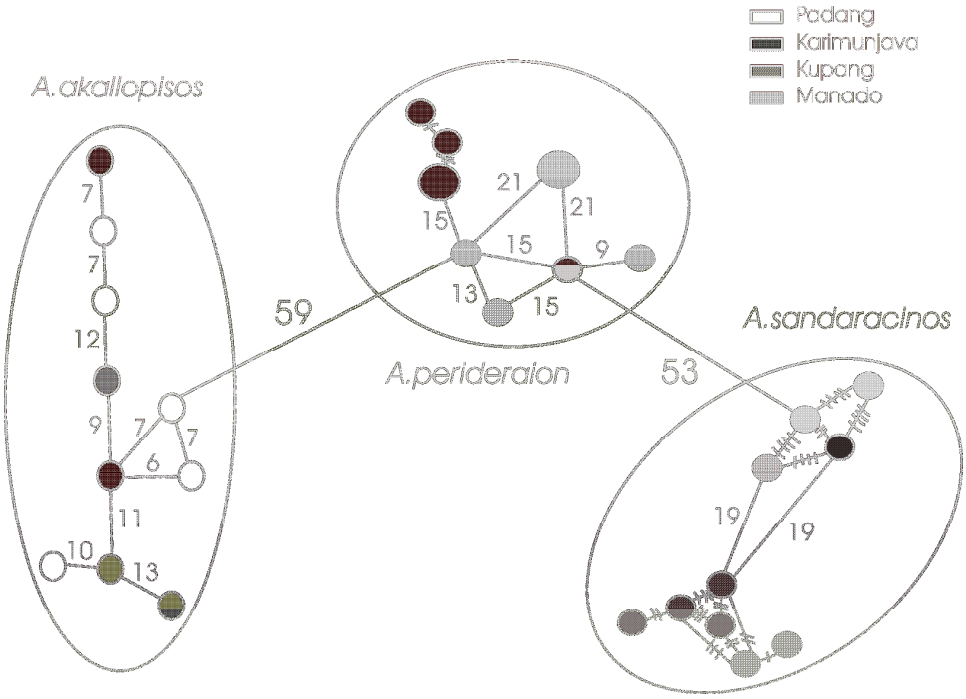


Figure 4

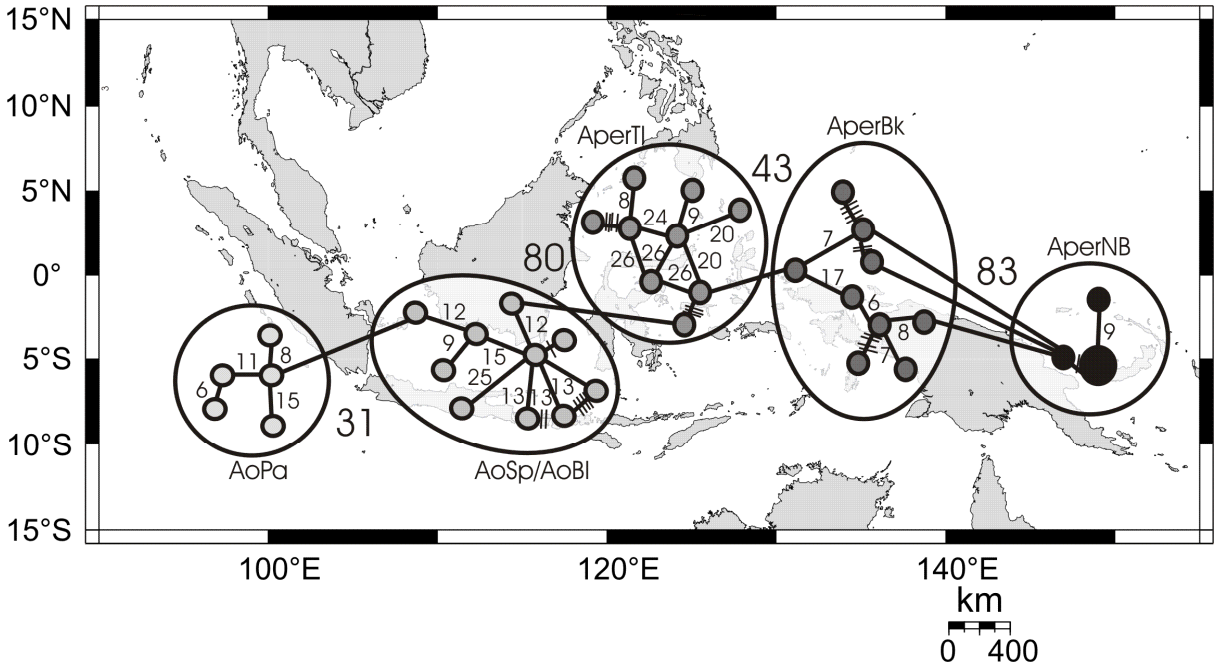


Figure 5

Strong genetic population structure in the boring giant clam *Tridacna crocea* across the Indo-Malay Archipelago

Marc Kochzius, Agus Nuryanto

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Abstract

Even though the Indo-Malay Archipelago hosts the world's greatest diversity of marine species, studies on the genetic population structure and gene flow of marine organisms within this area are rather rare. Consequently, not much is know about connectivity of marine populations in the Indo-Malay Archipelago, despite the fact that such information is important to understand evolutionary and ecological processes in the centre of marine biodiversity. This study aims to investigate the genetic population structure of the boring giant clam *Tridacna crocea*. The analysis is based on a 456 bp fragment of the cytochrome oxidase I gene from 300 individuals collected from 15 localities across the Indo-Malay Archipelago. *T. crocea* shows a very strong genetic population structure and isolation by distance, indicating restricted gene flow between almost all sample sites. The observed Φ_{st}-value of 0.28 is very high compared to other studies on giant clams. According to the pronounced genetic differences, the sample sites can be divided into four groups from West to East: (1) Eastern Indian Ocean (Padang), (2) Java Sea (Pulau Seribu, Karimunjava), (3) South China Sea (Kota Kinabalu), Indonesian throughflow (Manado, Sangalaki, Spermonde, Bira, Komodo, Kupang), as well as eastern coast of Sulawesi (Sembilan, Kendari, Luwuk, Togian Islands), and (4) Western Pacific (Biak). This complex genetic population structure and pattern of connectivity, characterised by restricted gene flow between some sites and panmixing between others can be attributed to the geological history and prevailing current regimes in the Indo-Malay Archipelago.

Keywords: aquarium trade, neutrality test, population expansion, conservation, marine protected areas, Southeast Asia

Introduction

Geology, genetic differentiation and evolution in the Indo-Malay archipelago

The Indo-Malay Archipelago, situated in a triangle between Sumatra, New Guinea, and the Philippines, hosts the world's greatest diversity of marine shallow water species. Several theories are proposed to explain the high diversity. They fall into three main categories: centre-of-evolutionary-radiation from where new species disperse (Briggs 1999), centre-of-overlap of the Indian and Pacific Oceans biota (Woodland 1983), and centre-of-accumulation of species that originated in peripheral areas (Jokiel and Martinelli 1992). Studies on molecular systematics of butterflyfishes (*Chaetodon* spp., McMillan and Palumbi 1995), lionfishes (*Pterois miles* and *P. volitans*, Kochzius *et al.* 2003), and starfishes (*Linckia laevigata* and *L. multifora*, Williams 2000) have shown a phylogenetic break between the Indian and Pacific Oceans, supporting the view of speciation in separated ocean basins.

Primary hypothesised triggers for this genetic separation of the two Ocean basins are plate tectonic movements in the Indo-Malay Archipelago and global fluctuation of sea level during multiple Pliocene and Pleistocene glaciations. About 30 million years ago most of the present islands in the Indo-Malay Archipelago did not exist or were not at their current position. For instance, the bird's head peninsula was not joined yet with New Guinea, which was still much further south, and Halmahera did not exist. Therefore, the Indian and Pacific Oceans were freely interconnected by the so-called Indonesian seaway that allowed the water masses of the Pacific South Equatorial Current (SEC) to enter the Indian Ocean. Due to the northward movement of Australia, New Guinea, and the Bird's head peninsula, as well as the development of Sulawesi by the amalgamation of several fragments, this current pattern started to change about 25 million years ago (Hall 1998). Especially the northward displacement of New Guinea about 5 million years ago had a major impact and 3-4 million years ago the Indonesian seaway was closed (Cane and Molnar 2001). The SEC was deflected, finally leading to the present situation that the waters of the Indonesian throughflow (ITF), which is the major exchange of water masses between the two oceans, originates from the northern Pacific (Gordon and Fine 1996, Gordon 2005).

Parallel to these tectonic events, multiple glaciations in the Pliocene and Pleistocene caused global fluctuations of the sea level with low stands of up to 120 m (Krantz 1991, Rohling *et al*. 1998, Siddall *et al*. 2003). Shallow shelf areas, such as the Sunda

shelf (Java Sea) between Java, Sumatra and Borneo, fell dry and separated ocean basins (Voris 2000, Figure 1), which caused allopatric speciation (McManus, 1985; Pandolfi, 1992; Benzie, 1998, 1999a; Randall 1998). Molecular genetic studies suggest as well that these separations have a Pliocene and Pleistocene origin (Kochzius et al. 2003, Barber *et al.* 2006).

A genetic break can also be observed in populations of damselfish (*Chromis viridis*, Froukh and Kochzius in press; *Stegastes nigricans*, *Chrysiptera biocellata*, *C. glauca* and *C. leucopoma*, Lacson and Clark 1995), clownfish (*Amphiprion ocellaris*, Nelson *et al.* 2000), seahorse (*Hippocampus spinosissimus*, Lourie et al. 2005), jobfish (*Lates calcarifer*, Chenoweth *et al.* 1998), starfish (*L. laevigata*, Williams and Benzie 1998, Williams *et al.* 2002; *Acanthaster planci*, Benzie 1999b), mantis shrimp (*Haptosquilla pulchella*, *H. glyptocercus*, and *Gonodactylellus viridis*, Barber *et al.* 2002, 2006), coconut crab (*Birgus latro*, Lavery *et al*. 1996), tiger prawn (*Penaeus monodon*, Duda and Palumbi 1999, Brooker *et al*. 2000, Sugama *et al*. 2002), mudcrab (*Scylla serrata*, Gopurenko et al. 1999), ginat clam (*Tridacna squamosa*, Kittiwattanawong *et al*. 2001), and a mangrove tree (*Avicennia marina*, Duke et al. 1998). The persistence of this genetic differentiation across the Indo-Malay Archipelago indicates that factors, such as restricted dispersal capabilities, ecological requirements, and current pattern prevent panmixing in these species.

However, such a genetic break between the Indian and Pacific Oceans is not a general pattern. Molecular phylogentic studies on marine intertidal snails have revealed that there is e.g. a phylogenetic break in two species from ecologically continental habitats (*Echinolittorina trochoides* clade A and *E. vidua*, Reid *et al.* 2006), whereas two species from ecologically oceanic habitats (E. *trochoides* clade D and *E. reticulata*) do not show such a break. Such a genetic break is also missing in a sea urchin (*Diadema savignyi*, Lessios *et al.* 2001), tasslefish (*Polynemus sheridani*, Chenoweth and Hughes 2003), bigeye tuna (*Thunnus obesus*, Alvarado Bremer *et al.* 1998, Chow *et al.* 2000), and swordfish (*Xiphias gladius*, Chow *et al.* 1997).

Connectivity of populations in the Indo-Malay Archipelago

Studies on the genetic population structure and gene flow of marine organisms within the Indo-Malay Archipelago are rather rare (Benzie 1998). Those few studies which have been conducted have investigated fishes, such as an anemonefish (Nelson *et al*.

2000), jobfish (Ovenden *et al*. 2004), seahorses (Lourie *et al.* 2005) as well as scad mackerels (Arnaud *et al*. 1999; Perrin and Borsa 2001, Borsa 2003, Rohfritsch and Borsa 2005), and a few invertebrates, such as mantis shrimps (Barber *et al*. 2002, Barber *et al.* 2006), and the giant tiger prawn (Sugama *et al*. 2002). Of these species, only mantis shrimps and seahorses are well covered, with many populations sampled across the archipelago. Even though several studies have dealt with the population genetics of giant clams (Ayala *et al*. 1973, Campbell *et al.* 1975, Benzie & Williams 1992a, b, Macaranas *et al*. 1992, Benzie & Williams 1995, Benzie & Williams, 1997, Kittiwattanawong 1997, Yu *et al*. 2000, Kittiwattanawong *et al.* 2001, Laurent *et al*. 2002, Juinio-Meñez *et al*. 2003, Ravago-Gotanco *et al.* 2007), none of them investigated populations within the Indo-Malay Archipelago on a large scale. Therefore, not much is known about connectivity of marine populations in the Indo-Malay Archipelago in general and especially for giant clams, despite the fact that such information is important to understand evolutionary and ecological processes in the centre of marine biodiversity. Data on connectivity are also valuable for conservation of these living marine resources.

Studied species and applied molecular marker

The boring or crocus clam *Tridacna crocea* is the smallest species of the giant clams (Tridacnidae). It grows to a length of about 15 cm and is characterised by bright colour pattern. As a sessile organism it bores into the substrate, such as coral boulders or reef rocks. The distribution of *T. crocea* ranges from the Eastern Indian Ocean across the Indo-Malay Archipelago to the Western Pacific (Lucas 1988). Like other giant clams, *T. crocea* hosts photosymbiotic dinoflagellates of the genus *Symbiodinium* (Hirose *et al.* 2006). These zooxanthellae transfer photosynthetically fixed carbon, mainly as glucose, to their host (Ishikura *et al.* 1999).

Connectivity of populations can be inferred by investigating the genetic population structure with molecular markers, such as cytochrome c oxidase I gene (COI). Studies on the population genetics of bivalves have shown that COI is a suitable marker for such studies (e.g. Luttikhuizen *et al*. 2003, Shefer *et al*. 2004).

This study aims to investigate the genetic population structure of the boring giant clam *T. crocea* across the Indo-Malay Archipelago based on COI sequences in order to study gene flow in the centre of marine biodiversity.

Materials and Methods

Sampling

Tissue sample from 300 specimens of *T. crocea* were collected during several field trips in 2004 and 2005 at 15 sample sites across the Indo-Malay Archipelago (Figure 1, Table 1). A small piece of mantel tissue was cut off from each specimen under water. This is a biopsy method that ensures the survival of the animal. Tissue samples were preserved in 96 % of ethanol and later stored at 4°C.

DNA extraction, amplification and DNA sequencing

Genomic DNA was isolated with the Chelex® method, following the protocol of Walsh *et al*. (1991). The molecular marker used in this study is the mitochondrial cytochrome c oxidase I (COI) gene. A fragment of this gene was amplified using the following tridacnid-specific primers, which were designed based on *T. crocea* sequences obtained in a preliminary analysis using of COI primer from Folmer *et al*. (1994): COI-Tricro-Frwd 5'-GGG TGA TAA TTC GAA CAG AA-3' and COI-Tricro-Rev 5'-TAG TTA AAG CCC CAG CTA AA-3' (reverse). The PCR reaction was carried out in a total volume of 50 µl. PCR reactions contained 1 µl DNA template, 10 mM Tris-HCl (pH 9), 50 mM KCl, 2 mM of $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP and 1 unit Taq polymerase. Thermal cycles were as follows: one cycle at 94°C for 3 minutes, followed by 35 cycles of 1 minute at 94°C, 1.5 minutes at 43°C, and 1 minute at 72°C. Final extension was conducted at 72°C for 5 minutes. The PCR products were purified using the QIA quick spin column PCR purification kit (Qiagen, Hilden, Germany) following the protocol from the manufacturer. The purified DNA was sequenced using the DyeDeoxy terminator chemistry (PE Biosystem, Foster City, USA) and an automated sequencer (ABI Prism 310, Applied Biosystems, Weiterstadt, Germany).

Genetic diversity

All sequences were edited with the programme Sequence Navigator (ver. 1.0.1; Applied Biosystem) and checked manually by eye. Orthology to a previous published sequence available in the European Molecular Biological Laboratory (EMBL).sequence data base (*T. crocea*, accession number AB076920) was also verified. No contamination with DNA from zooxanthellae was observed. The sequences were translated to amino acids with the programme Squint (http://www.cebl.auckland.ac.nz/index.php) in order to exclude mistakes in sequencing and to verify if a functional mitochondrial DNA sequence was obtained and not a nuclear pseudogene. A multiple sequences alignment was obtained by using ClustalW (Thompson *et al.* 1994) as implemented in the software Bioedit (ver. 7.0.4.1; Hall 1999) Haplotype diversity *h* (Nei 1987) and nucleotide diversity *π* (Nei & Jin 1989) were calculated with the programme Arlequin (ver. 2.0; Schneider *et al*. 2000).

Historical demography

The null hypothesis of neutral evolution of the marker was tested using Tajima's *D* test (Tajima 1989) and Fu's *Fs* test (Fu 1997) with 10,000 permutations as implemented in Arlequin. Mismatch distribution analysis and the model of sudden population expansion (Rogers 1995) was also tested using Arlequin based on 10,000 permutations.

Genetic population structure and connectivity

Significance of population structure was tested with Analysis of Molecular Variance (AMOVA; Excoffier *et al*. 1992) and pairwise Φ_{st}-values. Both statistical calculations were carried out using the software Arlequin (version 2.0, Schneider *et al*. 2000). A gamma shape parameter of 0.24 and the Tamura-Nei substitution model were used based on the result obtained with the programmes PAUP (ver. 4.0b10; Swofford 1998) and Modeltest (ver. 3.7; Posada & Crandall 1998). A haplotype network was also calculated with the programme Arlequin.

The correlation between geographic and genetic distances among populations was investigated using Reduced Major Axis (RMA) regression analysis and significance of the correlation was tested by a Mantel test using the program isolation by distance

web service (IBDWS 2.6) with 10,000 permutations to test for significance (Jensen *at al.* 2005; http://ibdws.sdsu.edu/). Geographic distances were measured with an electronic world atlas, distances representing the shortest path between two populations by sea.

Results

Genetic diversity

A sequences alignment of 456 bp of the COI fragment from 300 individuals of *T. crocea* collected from 15 localities across the Indo-Malay Archipelago (Table 1) was obtained. Among these 300 individuals a total number of 132 haplotypes was observed, with 117 polymorphic sites (25.7%) and 120 substitutions. The number of transitions was much higher than the number of transversions.

Genetic diversity within each population showed high levels of haplotype and nucleotide diversity, except for the populations from the Java Sea (Karimunjava and Pulau Seribu) and eastern coast of Sulawesi (Togian Islands, Luwuk, and Kendari). Haplotype diversity ranges from 0.60 in the population from Pulau Seribu to 1.00 in Manado, nucleotide diversity from 0.68% in Pulau Seribu to 4.34% in Biak (Table 1). Overall haplotype diversity (*h*) was 0.93 and nucleotide diversity (π) was 1.5 %.

Historical demography

Tajima's *D* test did not reject the null hypothesis of neutral evolution of the COI marker for most sample sites, whereas Fu's *Fs* test rejected the null hypothesis for most of the sites. However, this could indicate population expansion, which is supported by the mismatch distribution analysis and Rogers' test of sudden population expansion (Rogers 1995; Table 1).

Genetic population structure and connectivity

The evolutionary relationships among 132 *T. crocea* haplotypes are presented in the haplotype network (Figure 1), showing eight different clades. The distribution of clades across the Indo-Malay Archipelago is also presented on the map in Figure 1. Clade 1 and clade 5 are the most divergent, separated by 16 and 20 substitutions, respectively. Clade 1 occurs exclusively in the Eastern Indian Ocean (Padang) and no other clade could be observed at that sample site, whereas clade 5 was only found in

the Western Pacific (Biak). Clade 2 could be found at all sample sites (except Padang), but in higher proportions at some sites along the ITF (Manado, Sangalaki, Spermonde, and Bira). The proportion of clade 3 was rather low at all sites and it was not observed in the Western Pacific (Biak), Bira, Luwuk, and Padang. Clade 4 was present at the majority of the sample sites (except Padang and Manado) and was dominant in the Java Sea (Pulau Seribi and Karimunjava). Most of the sample sites showed a small proportion of clade 6, but this clade was not observed in Biak, Bira, Kupang, Manado, and Padang. Clade 7 could be found at all sample sites, except the two most western ones in Padang and Pulau Seribu. The highest proportions (> 50%) of clade 7 were observed along the eastern coast of Sulawesi (Kendari, Luwuk, Togian Islands), as well as in Komodo and Kupang. Clade 8 showed only a distribution in the central part of the Indo-Malay Archipelago (Bira, Kendari, Komodo, Kota Kinabalu, Kupang, Luwuk, Sembilan, and Spermonde), with a generally low proportion.

The most common haplotype from clade 7 was found in 23.3 % of all individuals and was unique for the eastern areas of Indonesia (from Makassar Strait eastward to New Guinea). The second most common haplotype from clade 4 occurred in a frequency of 9% and had a broader distribution, ranging from the Java Sea, Makassar Strait, and eastern coast of Sulawesi to Biak.

The observed genetic structure among *T. crocea* populations is verified by AMOVA and pairwise Φ_{st}-values. These analyses considered a gamma shape parameter of 0.24 as well as the Tamura-Nei substitution model and revealed a highly significant genetic structure among *T. crocea* populations across the Indo-Malay Archipelago ($\Phi_{st} = 0.28$; $p < 0.001$). However, no significant genetic structure was found between inner and outer shelf populations of the Spermonde Archipelago ($\Phi_{st} = 0.02$; $p = 0.21$). Pairwise Φ_{st}-values between sample sites revealed a very complex pattern of significant differences and homogeneity. The majority of pairwise comparisons showed significant Φ_{st}-values (74%), the remainder indicated panmixing (Table 2). Significant Φ_{st}-values range from 0.05 to 0.86. The population from Padang (Eastern Indian Ocean) is the most divergent population with Φ_{st}-value ranging from 0.57 to 0.87, followed by Biak (Western Pacific) with values ranging from 0.22 to 0.57.

Based on the pairwise analysis, a hierarchical AMOVA was carried out with different groupings (Table 3). The highest fixation index ($\Phi_{ct} = 0.5$) and variation (50.80 %) in the hierarchical AMOVA among groups showed the assignment of the sample sites to

the following four different regions: (1) Eastern Indian Ocean (Padang), (2) Java Sea (Pulau Seribu, Karimunjava), (3) South China Sea (Kota Kinabalu), Indonesian throughflow (Manado, Sangalaki, Spermonde, Bira, Komodo, Kupang), as well as eastern coast of Sulawesi (Sembilan, Kendari, Luwuk, Togian Islands), and (4) Western Pacific (Biak)..

Additionally, we found a positive correlations between genetic (F_{st}) and geographical distances ($r = 0.43$, $p \leq 0.004$, Figure 2), clearly showing isolation by distance. The genetically very divergent populations from the sample sites in Padang and Biak were excluded from the analysis.

Discussion

Genetic diversity

The high level of polymorphism in the COI fragment found suggests greatly restricted gene flow between populations of the boring giant clam *T. crocea*. Levels of genetic diversity are comparable with other studies on marine bivalves (e.g. Luttikhuizen *et al*. 2003, Shefer *et al*. 2004).

High genetic diversity in *T. crocea* was found in all populations, except the populations from the Java Sea (Pulau Seribu and Karimunjava) and sites from the eastern coast of Sulawesi (Kendari, Luwuk, and Togian Islands). Low genetic diversity in giant clam populations could be explained by three factors or a combination of the three. Low genetic diversity in the Java Sea could be due to a founder effect, which was caused by the re-colonisation of the Sunda Shelf as sea level rose after the last glacial. However, the low genetic diversity at the eastern coast of Sulawesi is difficult to explain by a founder effect. Due to the very narrow shelf around Sulawesi, only very small areas were exposed during low sea level stands in the last glacial (Voris 2000). Therefore, the low genetic diversity could not be due to re-colonisation.

Another factor could be overexploitation, because giant clams in the Java Sea have been heavily harvested for their shells. The Javanese shell industry in Jakarta and Jepura received giant clam shells from Pulau Seribu and Karimunjava, respectively. They have been used amongst others as material for the production of so-called "teraso" tiles for interior decoration. However, it is not reported which giant clam species are utilised in the shell industry (Pasaribu 1988, Wells 1997). It appears that populations of giant clams are also overexploited in the Togian Islands, but *T. crocea*

seems to be less affected (Allen & McKenna 2001). Therefore, it is difficult to estimate to which extend exploitation is responsible for the low genetic diversity.
The third factor could be a population bottleneck due to bleaching events in the Java Sea in 1983 (Pulau Seribu; Brown and Suharsono 1990, Hoeksema 1991) and 1998 (Pulau Seribu and Karimunjava, Wilkinson 2002) as well as eastern coast of Sulawesi (Togian and Banggai Islands, Allen & McKenna 2001, Wilkinson 2002). Since giant clams depend nutritionally on their zooxanthellate symbionts (Ishikura *et al.* 1999), the loss of zooxanthellae during bleaching events negatively affects their fitness (Leggat *et al.* 2003).

Historical demography
Tajima's *D* and Fu's *Fs* neutrality tests rejected the null hypothesis of neutral evolution of the utilised marker at some sample sites (Table 1). However, these tests cannot distinguish between selection and changes in population size. Mismatch distribution analysis and Rogers' test of sudden population expansion (Rogers & Harpending 1992, Rogers 1995) indicated demographic growth. Changes in population size could be explained by the reduction of habitat during sea level low stands and re-colonisation of new habitats after rising of the sea level (Figure 1). Loss of habitat resulted in population bottlenecks, whereas the availability of new habitats after the rise of the sea level at the end of the last glacial enabled growth of the reduced populations. This hypothesised impact of the quarternary ice ages can be e.g. detected in the genetic population structure of Pacific coral reef fish populations. Lagoonal species, which were impacted by the loss of their habitat during sea level low stands, show a lower genetic diversity than outer slope species that did not suffer from such a habitat loss (Fauvelot *et al.* 2003).

Genetic population structure and connectivity
T. crocea shows a very strong genetic population structure across the Indo-Malay Archipelago and isolation by distance, indicating restricted gene flow between almost all sample sites. The observed Φ_{st}-value of 0.28 is very high compared to other studies on giant clams. However, a comparison is difficult, because all other studies used allozymes as a genetic marker and differences in the biology of even closely related species, as well as different oceanographic conditions can have profound

effects on the population structure. Studies on *T. crocea* in the Philippines revealed F_{ST}-values of 0.066 (Juinio-Meñez *et al.* 2003) and 0.065 (Ravago-Gotanco *et al.* 2007). Values for other giant clams (*T. derasa*, *T. gigas*, and *T. maxima*) ranged from 0 to 0.156 in the Western Pacific (Benzie & Williams 1992a, b, Macaranas *et al.* 1992, Benzie & Williams 1995, Benzie & Willimas 1997).

According to the pronounced genetic differences, the sample sites can be divided into four groups from West to East (Table 3): (1) Eastern Indian Ocean (Padang), (2) Java Sea (Pulau Seribu, Karimunjava), (3) South China Sea (Kota Kinabalu), Indonesian throughflow (Manado, Sangalaki, Spermonde, Bira, Komodo, Kupang), as well as eastern coast of Sulawesi (Sembilan, Kendari, Luwuk, Togian Islands), and (4) Western Pacific (Biak).. Such genetic breaks in the transition of the Indian and Pacific Ocean was also found in populations of a starfish (Williams & Benzie 1998), mantis shrimp (Barber *et al*. 2002, 2006), and fishes (Chenoweth *et al*. 1998, Nelson *et al*. 2000, Lourie *et al.* 2005). The differentiation between populations of the Indian and Pacific Ocean is hypothesised to be due to low sea level during glacials, when huge ice shields in higher latitudes bound water and sea level was dropped up to 120 m. During those times, the Java Sea on the Sunda shelf between Java and Borneo was dry land. This created several more or less isolated ocean basins and restricted the exchange between the Indian and Pacific Oceans (McManus 1985, Voris, 2000). Persistence of such genetic pattern and additional differentiation could be caused by major prevailing currents.

A detailed comparison of the genetic structure with current pattern and results from other genetic studies in the region is conducted below for the four identified groups in *T. crocea*. However, comparison of the genetic population structure in *T. crocea* with other species is sometimes difficult due to differences in the geographic resolution of sampling.

Eastern Indian Ocean: The extremely high Φ_{st}-values of up to 0.87 raise the question if the population from Padang represents a cryptic species. However, based on current morphological taxonomy, the specimens from Padang belong to *T. crocea* (Lucas 1988). Further comparative studies with additional giant clam species and an integrated taxonomy approach (Dayrat 2005), including genetics, morphology and

ecology, would be needed to clarify if the population from Padang represents a cryptic species.

The genetic isolation of the population in Padang (Figure 1, Table 2) is most probably caused by the prevailing outflow of water masses from the Java Sea through the Sunda Strait into the Indian Ocean (Wyrtki 1961, Hendiarti *et al.* 2004). Even though the main current along the western coast of Sumatra are directed to the South-East, planktonic larvae of *T. crocea* probably can not enter the Java Sea through the Sunda Strait. Currents parallel along the southern coast of Java are seasonally changing and eastward currents are deflected at Sumba in a westward direction. Larvae from the Java Sea that enter the Indian Ocean through the Sunda Strait can not reach the western coast of Sumatra due to the south-eastern currents (Figure 1, Wyrtki 1961).

A genetic separation of populations in the Eastern Indian Ocean from other sites in the Indo-Malay Archipelago could also be observed in the seahorse *Hippocampus spinosissimus* (Lourie *et al.* 2005), the clownfish *Amphiprion ocellaris* (Nelson *et al.* 2000), the scad mackerel *Decapterus macrosoma* (Arnaud et al. 1999), and the mantis shrimps *Haptosquilla pulchella* (Barber *et al.* 2002, 2006). However, the seahorses *H. barbouri, H. kuda, and H. trimaculatus*, (Lourie *et al.* 2005) as well as the Indian scad mackerel *D. russelli* (Rohfritsch and Borsa 2005) did not show such a genetic separation of the Eastern Indian Ocean.

Java Sea: Populations from the Java Sea (Pulau Seribu and Karimunjava) were not statistically different from each other, indicating high gene flow and panmixing (Table 2). This could be due to seasonal changing currents in the Java Sea (Figure 1), which connect these populations that are more than 400 km apart. Furthermore, the pelagic larval duration of about nine days (Lucas 1988) seem to be sufficient for *T. crocea* to travel such distance mediated by currents. However, both populations showed different pattern of genetic differentiation to Kota Kinabalu in the South China Sea. The population from Pulau Seribu was not statistically different from Kota Kinabalu, while the differentiation of the population from Karimunjava to Kota Kinabalu was significant. Connectivity between the South China Sea and Java Sea could be facilitated by southward currents along the north-western coast of Borneo, which enter the Java Sea in February (Wyrtki 1961). The different pattern of genetic differentiation of the two populations from the Java Sea to the South China Sea can

be explained by isolation-by-distance (Figure 2). Both populations in the Java Sea are significantly different to all the other sites from the Makassar Strait to the East (Table 2). Even though seasonal easterly currents could connect the Java Sea to the Makassar Strait and Flores Sea, isolation-by-distance seems to play a role.
Genetically distinct populations in the Java Sea were also present in the mantis shrimp *Gonodactylellus viridis* (Barber *et al.* 2006), but could not be detected in the seahorses *H. kuda*, *H. spinosissimus*, and *H. trimaculatus* (Lourie *et al.* 2005), as well as the Indian scad mackerel *D. russelli* (Rohfritsch and Borsa 2005). However, the genetic break observed in *T.crocea* in the eastern Java Sea (Figure 1, Table 2) also occurred in the seahorse *H. trimaculatus* (Lourie *et al.* 2005) and Indian scad mackerel *D. russelli* (Rohfritsch and Borsa 2005).

South China Sea, Indonesian throughflow, and eastern coast of Sulawesi: Pairwise comparisons of populations from the South China Sea at the north-western coast of Borneo (Kota Kinabalu), Sulawesi Sea at the north-eastern coast of Borneo (Sangalaki) and northern coast of Sulawesi (Manado), as well as Makassar Strait (Spermonde) and Flores Sea (Bira) at the south-western tip of Sulawesi did not show significant Φ_{st}-values, indicating panmixing. Connectivity of the South China Sea to the Sulawesi Sea could be facilitated by north-easterly currents around the northern tip of Borneo into the Sulu Sea and southward currents from the Sulu Sea into the Sulawesi Sea in October (Wyrtki 1961).
Sites in the Sulawesi Sea and Makassar Strait down to the south-eastern tip of Sulawesi in the Flores Sea are well connected by the ITF, which transports about 10 million m^3 of water per second (= 10 sverdrup; Godfrey 1996, Gordon and Fine 1996, Godfrey and Masumoto 1999, Gordon 2005, Susanto and Gordon 2005), reaching surface currents of up to 36 nautical miles per day (Wyrtki 1961). Connectivity of populations facilitated by the ITF can also be observed among sites bordering the Flores Sea (Bira and Komodo) as well as Kupang (Timor), which show no significant genetic differentiation (Table 2). However, Komodo and Kupang were significant different from sites upstream the ITF (Spermonde, Sangalaki, and Manado). This pattern of genetic differentiation along the ITF is most probably due to isolation-by-distance (Figure 2). One branch of the ITF flows from the Makassar Strait eastward through the Flores Sea into the Banda Sea, were it changes its direction and flows to

the South-West, reaching Kupang and Komodo (Figure 1, Gordon 2005). The genetic structure suggests that the populations in Kupang and Komodo are connected along this route to populations upstream the ITF, rather than being directly connected to the south-western tip of Sulawesi (Spermonde and Bira) across the Flores Sea. This is supported by the significant difference of Kupang and Komodo to Spermonde (Table 2).

A hierarchical AMOVA did not support a separate grouping of the sites in Bone Bay (Sembilan) and at the eastern coast of Sulawesi (Kendari, Luwuk, and Togian Islands). However, these sites are not significant different from each other and pairwise comparisons showed low, but significant Φ_{st}-values to all other sites, except Komodo and Kupang that are also not significantly different from each other (Table 2). It seems that sites at the eastern coast of Sulawesi (Kendari, Luwuk, and Togian Islands) are well connected and linked via Kendari at the south-eastern tip of Sulawesi to the ITF, which facilitates connectivity to Kupang and Komodo further downstream (Figure 1).

A genetic break between the western and eastern coast of Sulawesi was observed in Bone Bay. Populations in Bira and Sembilan are genetically significant different (Φ_{st} = 0.13), even though the geographic distance is only 67 km. Oceanographic conditions and the influence of different current regimes obviously prevent an exchange between the western and eastern coast of the island. Selayar, an island south of Bira at the south-western tip of Sulawesi, stretches along a North-South axis and is separated from Sulawesi only be a small strait. Therefore, the ITF coming from the Makassar Strait is deflected further south into the Flores Sea. However, lack of significant genetic differentiation suggests connectivity of the site in Bone Bay to the eastern coast of Sulawesi by currents.

Connectivity of sites at the north-western coast of Borneo in the South China Sea to sites along the ITF and eastern Sulawesi was also revealed in the seahorses *H. barbouri* and *H. trimaculatus* (Lourie *et al.* 2005). Panmixing at sites in the Sulwesi Sea and Makassar Strait along the ITF was also found in the Indian scad mackerel *D. russelli* (Rohfritsch and Borsa 2005), the clownfish *A. ocellaris* (Nelson *et al.* 2000), as well as the mantis shrimps *H. pulchella*, *H. glyptocercus*, and *G. viridis* (Barber *et al.* 2006). Tomini Bay was also not isolated in a study on the scad mackerel *D. macarellus*, which showed not genetic heterogeneity to the Moluccas (Arnaud *et al.*

1999). However, a study on the mantis shrimp *H. pulchella* revealed a strong genetic isolation of the population from Tomini Bay (Barber *et al.* 2002). No separation of the eastern and western coast of Sulawesi was observed in the two mantis shrimp species *H. glyptocercus* and *G. viridis* (Barber *et al.* 2006). These differences between species could be attributed to different dispersal capabilities of early life stages and adults.

Western Pacific: The population from Biak in the Western Pacific showed restricted gene flow to all other sites, with a genetic break somewhere in the Moluccas. The strong separation of Biak off the north-western coast of New Guinea could be caused by the Halmahera eddy, which redirects the westward SEC into the North Equatorial Counter Current (NECC) to the East (Figure 1, Wyrtki 1961, Gordon and Fine 1996). A genetic separation of Western Pacific sites was also found in the above mentioned three species of mantis shrimps, with a genetic break occurring in the Moluccas (Barber *et al.* 2006).

The complex genetic population structure and pattern of connectivity, characterised by restricted gene flow between some sites and panmixing between others can be attributed to the geological history and prevailing current regimes in the Indo-Malay Archipelago. The major observed genetic differentiation between the Indian Ocean and Western Pacific is most probably due to historical isolation by sea level changes, whereas current oceanographic conditions facilitate connectivity along the ITF and separation at sometimes very small scales, such as in Bone Bay. Prevailing current regimes at the western coast of Sumatra as well as the Halmahera eddy off north-western New Guinea prevent connectivity of populations in the Eastern Indian Ocean and Western Pacific to the central Indo-Malay Archipelago. These factors cause vicariance between populations, which can lead to allopatric speciation, suggesting that the Indo-Malay Archipelago is a centre-of-evolutionary-radiation (Briggs 1999).

Conservation

Giant clams are exploited for food, shells and aquarium trade on a local and international scale. According to CITES data, international trade in non-captive bred giant clams increased from about 40,000 specimens in 1993 to about 100,000 in 2001 (Wabnitz *et al*. 2003). Especially in Southeast Asia, stocks have been severely

overexploited, including most parts of the Indonesian Archipelago. All tridacnid species are listed in Appendix II of CITES, allowing international trade only with appropriate export permits. Even though giant clams are fully protected in Indonesia and collection, sale and export of wild specimens is banned, enforcement of this legislation is poor (Wells 1997). Clam meat has been consumed extensively by the coastal population in Indonesia and shells were used for different purposes, such as basins, ornaments, and production of tiles (Pasaribu 1988, Wells 1997). Due to their bright colouration pattern, *T. crocea* is a popular species in the aquarium trade (Wabnitz *et al*. 2003). In the 1990s an Indonesian company was reported to export over 100,000 wild caught, live specimens of *T. crocea* to the international market. It is known from the Philippines that collection causes severe reef damage, because corals are destroyed to extract the clams (Wells, 1997). Main importers for giant clams in the marine ornamental trade are the European Union and the United States of America with about 48,000 and 45,000 specimens imported in 2001, respectively (Wabnitz *et al*. 2003).

Marine protected areas (MPAs) are considered an important tool to prevent overexploitation and ensure the sustainable use of living marine resources (Palumbi 2001). Therefore, MPAs are strongly recommended to manage Indonesia's marine fisheries (Mous *et al*. 2005). It is proposed that MPAs should be arranged in a network and that the spatial distribution should match the dispersal capabilities of the species to be protected (Palumbi 2003).

The strong genetic population structure of *T. crocea* across the Indo-Malay Archipelago has important implications for the conservation of this species. The four defined groups should be managed separately, because exchange between the regions is limited. Detected small scale genetic differentiations in some regions suggest limited larval dispersal, which should be considered in the spatial arrangement of MPAs. Therefore, the Indonesian national system of about 51 MPAs covering 58,000 km^2 (Spalding *et al.* 2001) seems to be not sufficient to protect species with limited larval dispersal, because these MPAs only cover about 1 % of the total marine area of the Indonesian archipelago. However, further studies on other invertebrates and fishes are needed to draw a generalised picture of connectivity among populations in Indonesia and the Indo-Malay Archipelago in order to design a functional network of MPAs.

References

Alvarado Bremer JR,Stequert B, Robertson NW, Ely B (1998) Genetic evidence for inter-oceanic subdivision of bigeye tuna (*Thunnus obesus* Lowe) populations. *Marine Biology*, **132**, 547-557.

Allen GR, McKenna SA (2001) A marine rapid assessment of the Togean and Banggai Islands, Sulawesi, Indonesia. RAP Bulletin of Biological Assessment 20, Conservation International, Washington, DC.

Arnaud S, Bonhomme F, Borsa P (1999) Mitochondrial DNA analysis of the genetic relationships among populations of scad mackerel (*Decapterus macarellus*, *D. macrosoma*, and *D. russelli*) in South-East Asia. *Marine Biology*, **135**, 699-707.

Ayala FJ, Hedgecock D, Zumwalt GS, Valentine JW (1973) Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolutions*, **27**, 177-191.

Barber PH, Erdmann MV, Palumbi SR (2006) Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution,* **60**, 1825-1839.

Barber PH, Palumbi SR, Erdmand MV, Moosa MK (2002) Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes and consequences. *Molecular Ecology*, **11**, 659-674.

Benzie JAH (1998) Genetic structure of marine organisms and SE Asian biogeography. In: Biogeography and geological evolution of SE Asia (eds. Hall R and Holloway JD), pp. 197-209, Backhuys Publishers, Leiden.

Benzie JAH (1999a) Genetic structure of reef organisms: ghost dispersal of the past. *American Zoologist*, **39**, 131-145.

Benzie JAH (1999b) Major genetic differences between crown-of-thorns starfish (*Acanthaster planci*) populations in the Indian and Pacific Oceans. *Evolution*, 53, 1782-1795.

Benzie JAH, Williams ST (1992a) Genetic structure of giant clam (*Tridacna maxima*) populations from reefs in the Western Coral Sea. *Coral Reefs*, **11**, 135-141.

Benzie JAH, Williams ST (1992b) No genetic differentiation of giant clam (*Tridacna gigas*) populations in the Great Barrier Reef, Australia. *Marine Biology*, **112**, 1-5.

Benzie JAH, Williams ST (1995) Gene flow among giant clam (*Tridacna gigas*) populations in Pacific does not parallel ocean circulation. *Marine Biology*, **123**, 781-787.

Benzie JAH, Williams ST (1997) Genetic structure of giant clam (*Tridacna maxima*) populations in the west Pacific is not consistent with dispersal by present-day ocean currents. *Evolution*, **51**, 768-783

Borsa P (2003) Genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) in the Indo-Malay Archipelago. *Marine Biology*, **142**, 575-581.

Briggs JC (1999) Coincident biogeographic patterns: Indo-West Pacific Ocean. *Evolution*, **53**, 326-335.

Brooker AL, Benzie JAH, Blair D, Versini JJ (2000) Population structure of the giant tiger prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. *Marine Biology*, **136**, 149–157.

Brown BE, Suharsono (1990) Damage and recovery of coral reefs affected by El Niño related seawater warming in the Thousand Islands, Indonesia. *Marine Biology*, **8**, 163-170.

Campbell CA, Valentine JW, Ayala FJ (1975) High genetic variability in a population of *Tridacna maxima* from the Great Barrier Reef. *Marine Biology*, **33**, 341-345.

Cane MA, Molnar P (2001) Closing of the Indonesian seaway as a precursor to east African aridification around 3-4million years ago. *Nature*, **411**, 157-162.

Chenoweth SF, Hughes JM (2003) Oceanic interchange and nonequilibrium population structure in the estuarine dependent Indo-Pacific tasselfish, *Polynemus sheridani*. *Molecular Ecology*, **12**, 2387-2397.

Chenoweth SF, Hughes JM, Keenan CP, Lavery S (1998) When oceans meet: a teleost shows secondary integration at an Indian-Pacific interface. *Proceedings of the Royal Society of London B*, **265**, 415-420.

Chow S, Okamoto H, Uozumi Y, Takeuchi Y, Takeyama H (1997) Genetic stock structure of the swordfish (*Xiphias gladius*) inferred by PCR-RFLP analysis of the mitochondrial DNA control region. *Marine Biology*, **127**, 359-367.

Chow S, Okamoto H, Miyabe N, Hiramatsu K, Barut N (2000) Genetic divergence between Atlantic and Indo-Pacific stocks of bigeye tuna (*Thunnus obesus*) and admixture around South Africa. *Molecular Ecology*, **9**, 221-227.

Clement M, Posada D, Crandall KA (2000) TCS: a program to estimate gene genealogies. *Molecular Ecology*, **9**, 1657-1660.

Dayrat B (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society*, **85**, 407-415.

Duda TF Jr., Palumbi SR (1999) Population structure of the black tiger prawn, *Penaeus monodon*, among western Indian Ocean and western Pacific populations. *Marine Biology*, **134**, 705-710.

Duke NC, Benzie JAH, Goodall JA, Ballment ER (1998) Genetic structure and evolution of species in the mangrove genus *Avicennia* (Avicenniaceae) in the Indo-West Pacific. *Evolution*, **52**, 1612-1626.

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**, 479-491.

Fauvelot C, Bernardi G, Planes S (2003) Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from holocene sea-level change. *Evolution*, **57**, 1571-1583.

Folmer O, Black M, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3,** 294-299.

Fu FX (1997) Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics*, **147**, 915-925.

Froukh T, Kochzius M (in press) Species boundaries and evolutionary lineages in the blue green damselfishes *Chromis viridis* and *C. atripectoralis* (Pomacentridae). *Journal of Fish Biology*

Godfrey JS (1996) The effect of the Indonesian throughflow on ocean circulation and heat exchange with the atmosphere: A review. *Journal of Geophysical Research*, **101**, 12,217-12,238.

Godfrey JS, Masumoto (1999) Diagnosing the mean strength of the Indonesian Throughflow in an ocean general circulation model. *Journal of Geophysical Research*, **104**, 7889-7895.

Gopurenko D, Hughes JM, Keenan CP (1999) Mitochondrial DNA evidence for rapid colonisation of the Indo-West Pacific by the mudcrab *Scylla serrata*. *Marine Biology*, **134**, 227-233.

Gordon AL (2005) Oceanography of the Indonesian seas and their throughflow. *Oceanography*, **18**, 14-27.

Gordon AL, Fine RA (1996) Pathways of water between the Pacific and Indian oceans in the Indonesian seas. *Nature*, **379**, 146-149

Hall R (1998) The plate tectonics of cenozoic SE Asia and the distribution of land and sea. In: Biogeography and geological evolution of SE Asia (eds. Hall R and Holloway JD), pp. 99-131, Backhuys Publishers, Leiden.

Hall TA (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series*, **41**, 95-98.

Hendiarti N, Siegel H, Ohde T (2004) Investigation of different coastal processes in Indonesian waters using SeaWiFS data. *Deep-Sea Research Part II*, **51**, 85-97.

Hirose E, Adachi R, Kuze K (2006) Establishment of the photosymbiosis in the early ontogeny of three giant clams. *Marine Biology*, **148**, 551-558.

Hoeksema BW (1991) Control of bleaching in mushroom coral populations (Scleractinia: Fungiidae) in the Java Sea: stress tolerance and interference by life history strategy. *Marine Ecology Progress Series*, **74**, 225–237.

Ishikura M, Adachi K, Maruyama T (1999) Zooxanthellae release glucose in the tissue of a giant clam, *Tridacna crocea*. *Marine Biology*, **133**, 665-673.

Jensen JL, Bohonak AJ, Kelley ST (2005) Isolation by distance, web service. *BMC Genetics*, **6**, 13.

Jokiel P, Martinelli FJ (1992) The vortex model of coral reef biogeography. *Journal of Biogeography*, **19**, 449-458.

Juinio-Meñez MA, Magsino RM, Ravago-Gotanco R, Yu ET (2003) Genetic structure of *Linckia laevigata* and *Tridacna crocea* populations in the Palawan shelf and shoal reefs. *Marine Biology*, **142**, 717-726.

Kittiwattanawong K (1997) Genetic structure of giant clam, *Tridacna maxima* in the Andaman Sea, Thailand. *Phuket Marine Biological Center Special Publication* **17**, 109–114.

Kittiwattanawong K, Nugranad J., Srisawat T. (2001) High genetic divergence of *Tridacna squamosa* living at the west and east coasts of Thailand. *Phuket Marine Biological Center Special Publication* **25**, 343–347.

Kochzius M, Söller R, Khalaf MA, Blohm D (2003) Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution* **28**, 396-403.

Krantz DE (1991) A chronology of Pliocene sea-level fluctuations: the U.S. middle Atlantic costal plain record. *Quaternary Science Reviews*, **10**, 163-174

Lacson JM, Clarke S (1995) Genetic divergence of Maldivian and Micronesian demes of the damselfishes *Stegastes nigricans*, *Chrysiptera biocellata*, *C. glauca* and *C. leucopoma* (Pomacentridae). *Marine Biololgy*, **121**, 585-590.

Laurent V, Planes S, Salvat B (2002) High variability of genetic pattern in giant clam (*Tridacna maxima*) populations within French Polynesia. *Biological Journal of the Linnaean Society*, **77**, 221-231.

Lavery S, Moritz C, Fielder DR (1996) Indo-Pacific population structure and evolutionary history of the coconut crab *Birgus latro*. *Molecular Ecology*, **5**, 557-570.

Leggat W, Buck BH, Grice A, Yellowlees D (2003) The impact of bleaching on the metabolic contribution of dinoflagellate symbionts to their giant clam host. *Plant, Cell and Environment*, **26**, 1951-1961.

Lessios HA, Kessing BD, Pearse JS (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin *Diadema*. *Evolution*, **55**, 955-975.

Lourie SA, Green DM, Vincent ACJ (2005) Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). *Molecular Ecology*, 14, 1073-1094

Lucas JS (1988) Giant clams: description, distribution and life history. In: Giant Clams in Asia and the Pcific (eds. Copland JW, Lucas JS), pp. 21-32. Australian Centre for International Agricultural Research, Canberra.

Luttikhuizen PC, Drent J, Baker AJ (2003) Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in marine bivalve with pelagic larval dispersal. *Molecular Ecology*, **12**, 2215-2229.

Macaranas JM, Ablan CA, Pante MJR, Benzie JAH, Williams STH (1992) Genetic structure of giant clam (*Tridacna derasa*) populations from reefs in the Indo-Pacific. *Marine Biology*, **113**, 231-238.

McManus JW (1985) Marine speciation, tectonics and sea-level changes in Southeast Asia. *Proceedings of the Fifth International Coral Reef Congress*, Tahiti **4**, 133–138.

McMillan WO, Palumbi SR (1995). Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. *Proceedings of the Royal Society of London B*, **260**, 229-236.

Mous PJ, Pet JS, Arifin Z, Djohani R, Erdmann MV, Halim A, Knight H, Pet-Soede L, Wiadnya G (2005) Policy needs to improve marine capture fisheries management and to define a role for marine protected areas in Indonesia. *Fisheries Management and Ecology*, **12**, 259-268.

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York.

Nei M, Jin L (1989) Variances of the average numbers of nucleotide substitutions within and between populations. *Molecular Biology and Evolution*, ***6***, 290-300.

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000) Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. *Marine Biology*, **137**, 727-736.

Ovenden JR, Salini JS; O'Connor, Street R (2004) Pronounced genetic population structure in a potentially vagile fish species (*Pristipomoides multidens*, Teleostei; Perciformes, Lutjanidae) from the East Indies triangle. *Molecular Ecology*, **13**, 1991-1999.

Palumbi SR (2001) The ecology of marine protected areas. In: Marine Community Ecology (eds. Bertness MD, Gaines SM, Hixon ME), pp. 509-530. Sinauer Associates, Sunderland.

Palumbi SR (2003) Population genetics, demographic connectivity, and the design of marine reserves. *Ecological Applications*, **13**, S146-S158.

Pandolfi JM (1992) Successive isolation rather than evolutionary centres for the origination of Indo-Pacific reef corals. Journal of Biogeography 19: 593-609.

Pasaribu BP 1988. Status of giant clams in Indonesia. In: Giant Clams in Asia and the Pacific (eds. Copland JW, Lucas JS), pp. 44-46. Australian Centre for International Agricultural Research, Canberra.

Perrin C, Borsa P (2001) Mitochondrial DNA analysis of the geographic structure of Indian scad mackerel in the Indo-Malay archipelago. *Journal of Fish Biology*, **59**, 1421-1426.

Posada D, Crandall KA (1998) Modeltest: testing the model of DNA substitution. *Bioinformatics*, **14**, 817-818.

Randall JE (1998) Zoogeography of shore fishes of the Indo-Pacific region. *Zoological Studies* 37(4): 227-268.

Ravago-Gotanco RG, Magsino RM, Juinio-Menez MA (2007) Influence of the North Equatorial Current on the population genetic structure of *Tridacna crocea* (Mollusca: Tridacnidae) along the eastern Philippine seaboard. *Marine Ecology Progress Series*, **336**, 161-168.

Reid DG, Lal K, Mackenzie-Dodds J, Kaligis F, Littlewood DTJ, Williams ST (2006). Comparative phylogeography and species boundaries in *Echinolittorina* snails in the central Indo-West Pacific. *Journal of Biogeography*, **33**, 990-1006.

Rogers AR (1995) Genetic evidence for Pleistocene population expansion. *Evolution*, **49**, 608-615.

Rogers A, Harpending H (1992) Population growth makes in the distribution of pairwise genetics differences. *Molecular Biology and Evolution*, **9**, 552-569.

Rohfritsch A, Borsa P (2005) Genetic structure of Indian scad mackerel *Decapterus russelli*: Pleistocene vicariance and secondary contact in the Central Indo-West Pacific Seas. *Heredity*, **95**, 315–326

Rohling EJ, Fenton M, Jorissen FJ, Bertrand P, Ganssen G, Caulet JP (1998) Magnitudes of sea-level lowstands of the past 500,000 years. *Nature*, **394**, 162-165.

Schneider S, Roessli D, Excoffier L (2000) Arlequin, version 2.000. University of Geneva, Geneva.

Shefer S, Abelson A, Mokady O, Geffen E (2004) Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or antrophogenic transport? *Molecular Ecology*, **12**, 2333-2343.

Siddall M, E. Rohling J, Almogi-Labin A, Hemleben Ch, Meischner D, Schmelzer I, Smeed DA (2003) Sea-level fluctuations during the last glacial cycle. *Nature*, **423**, 853-858.

Spalding MD, Ravilious C, Green EP (2001) World Atlas of Coral Reefs. UNEP World Conservation Monitoring Centre. University of California Press, Berkeley.

Sugama K, Haryanti, Benzie JAH, Ballment E (2002) Genetic variation and population structure of the giant tiger prawn, *Penaeus monodon*, in Indonesia. *Aquaculture*, **205**, 37-48.

Susanto RD, Gordon AL (2005) Velocity and transport of the Makassar Strait throughflow, *Journal of Geophysical Research*, **110**, C01005

Swofford DL (1998) PAUP*. Phylogenetic analysis using parsimony (* and other methods). Version 4.0b10. Sinauer, Sunderland.

Tajima F (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, **123**, 585-595.

Thompson JG, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673–4680.

Voris HK (2000) Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time duration. *Journal of Biogeographie*, **27**, 1153–1167.

Wabnitz C, Taylor M, Green E, Razak T (2003) From ocean to aquarium. UNEP-WCMC. Cambridge.

Walsh PS, Metzger DA, Higushi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**, 506-513.

Wells S (1997) Giant clams: status, trade and mariculture, and the role of CITES management. IUCN, Gland, Switzerland, and Cambridge.

Wilkinson C (2002) Status of coral reefs of the world: 2002. Australian Institute of Marine Science.

Williams ST (2000) Species boundaries in the starfish genus *Linckia*. Marine Biology, **136**, 137-148.

Williams ST, Benzie JAH (1998) Evidence of a biogeographic break between populations of a high dispersal starfish: congruent regions within the Indo-West Pacific defined by color morphs, mtDNA, and allozyme data. *Evolution* **52**, 87-99

Williams ST, Jara J, Gomez N, Knowlton N (2002) The marine Indo-West Pacific break: contrasting the resolving power of mitochondrial and nuclear genes. *Integrated Comparative Biology* **42**, 941-952.

Wyrtki K (1961) Physical oceanography of the Southeast Asian waters. University of California, La Jolla.

Woodland DJ (1983) Zoogeography of the Siganidae (Pisces): An interpretation of distribution and richness pattern. *Bulletin of Marine Science* **33**, 713-717.

Yu ET, Juinio-Meñez MA, Monje VD (2000) Sequence variation in the ribosomal DNA internal transcribed spacer of *Tridacna crocea*. *Marine Biotechnology*, **2**, 511-516.

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Figure Legends

Figure 1 A. Map of the Indo-Malay Archipelago showing samples sites (for abbreviations see Table 1) as well as oceanographic pattern with dominant (solid lines) and seasonally changing (dashed lines) currents (Wyrtki 1961, Gordon and Fine 1996, Gordon 2005; *ITF*: Indonesian throughflow, SEC: Southern equatorial current, NECC: Northern Equatorial Counter Current). Pleistocene maximum sea level low stand of 120 m is indicated by the light grey area (Voris 2000). Pie charts represent the proportion of clades defined in the network at the different sample sites. B. Network of 132 mitochondrial cytochrome c oxidase I haplotypes from 300 individuals of *Tridacna crocea*. Connecting lines between circles represent one mutational step. The hatches and numbers indicate additional mutational steps. The size of the circles is proportional to haplotype frequency.

Figure 2 Relationship between genetic versus geographic distances in *Tridacna crocea* using Reduced Major Axis (RMA) regression without considering divergent populations from Padang and Biak.

Table 1 Sample sites, number of sequences (n), number of halpotypes (Nhp), haplotype diversity (*h*), nucleotide diversity (π), Tajima's D, Fu's Fs, sum od square deviation (SSD), and Harpending's Raggedness index (HRI) for *Tridacna crocea* in the Indo-Malay Archipelago. * = 0.05 ≥ *p* ≥ 0.01, ** = 0.01 > *p* ≥ 0.001, *** = *p* < 0.001; ns = not significant.

			Genetic diversity			Neutrality tests		Mismatch distribution	
Code	Sample site	n	Nhp	*h*	π (%)	Tajima's D	Fu's Fs	SSD	HRI
Bi	Bira, Sulawesi	12	8	0.91 ± 0.065	0.89 ± 0.54	-0.51^{ns}	-1.84^{ns}	0.03^{ns}	0.06^{ns}
Bk	Biak, New Guinea	14	13	0.99 ± 0.031	4.34 ± 2.29	-0.21^{ns}	-2.84^{ns}	0.04^{ns}	0.02^{ns}
Ka	Karimunjava, Java	16	9	0.77 ± 0.113	0.69 ± 0.42	-1.71^{*}	-2.65^{*}	0.01^{ns}	0.04^{ns}
Ke	Kendari, Sulawesi	28	16	0.83 ± 0.074	0.79 ± 0.46	-1.73^{*}	-7.84^{***}	0.00^{ns}	0.01^{ns}
KK	Kota Kinabalu, Borneo	21	16	0.97 ± 0.023	1.14 ± 0.64	-1.39^{ns}	-7.98^{***}	0.00^{ns}	0.02^{ns}
Ko	Komodo	26	17	0.89 ± 0.059	1.01 ± 0.57	-2.12^{**}	-8.11^{***}	0.02^{ns}	0.03^{ns}
Ku	Kupang, Timor	9	7	0.92 ± 0.092	0.70 ± 0.45	-1.52^{ns}	-2.55^{*}	0.02^{ns}	0.08^{ns}
Lu	Luwuk, Sulawesi	23	13	0.82 ± 0.082	0.73 ± 0.44	-1.57^{*}	-4.50^{**}	0.00^{ns}	0.01^{ns}
Ma	Manado, Sulawesi	8	8	1.00 ± 0.063	0.85 ± 0.54	-0.66^{ns}	-4.89^{**}	0.01^{ns}	0.06^{ns}
Pa	Padang, Sumatra	7	6	0.95 ± 0.096	1.03 ± 0.66	-0.79^{ns}	-1.49^{ns}	0.04^{ns}	0.09^{ns}
PS	Pulau Seribu, Java	14	6	0.60 ± 0.150	0.68 ± 0.42	-0.67^{ns}	-0.02^{ns}	0.08^{ns}	0.21^{ns}
Sa	Sangalaki, Borneo	16	13	0.97 ± 0.036	1.31 ± 0.74	-0.89^{ns}	-5.35^{**}	0.01^{ns}	0.02^{ns}
Se	Sembilan, Sulawesi	20	14	0.94 ± 0.043	1.28 ± 0.72	-1.45^{ns}	-4.69^{*}	0.01^{ns}	0.02^{ns}
Sp	Spermonde, Sulawesi	40	24	0.96 ± 0.014	1.05 ± 0.58	-1.43^{ns}	-13.83^{***}	0.00^{ns}	0.01^{ns}
TI	Togian Islands, Sulawesi	46	21	0.79 ± 0.063	0.71 ± 0.41	-1.88^{*}	-12.16^{***}	0.01^{ns}	0.03^{ns}

Table 2 Pairwise Φ_{st}-values between populations of *Tridacna crocea* in the Indo-Malay Archipelago. For abbreviations of sample sites see Table 1. * = 0.05 ≥ *p* ≥ 0.01, ** = 0.01 > *p* ≥ 0.001, *** = *p* < 0.001; ns = not significant.

	Pa	PS	Ka	KK	Sa	Sp	Bi	Se	Ke	Lu	TI	Ma	Ko	Ku
PS	0.85***													
Ka	0.85***	-0.03 ^{ns}												
KK	0.81***	0.05 ^{ns}	0.06*											
Sa	0.80***	0.07*	0.09**	-0.02 ^{ns}										
Sp	0.82***	0.07*	0.08**	-0.02 ^{ns}	-0.02 ^{ns}									
Bi	0.84***	0.17**	0.18***	0.01 ^{ns}	-0.01 ^{ns}	-0.01 ^{ns}								
Se	0.80***	0.20***	0.20***	0.05*	0.07*	0.09**	0.13**							
Ke	0.85***	0.24***	0.21***	0.07**	0.10**	0.09***	0.11**	0.02 ^{ns}						
Lu	0.86***	0.26***	0.23***	0.06*	0.08*	0.07**	0.08*	0.04 ^{ns}	-0.02 ^{ns}					
TI	0.87***	0.27***	0.24***	0.08**	0.12***	0.10***	0.12***	0.04 ^{ns}	-0.01 ^{ns}	-0.01 ^{ns}				
Ma	0.83***	0.12*	0.14**	-0.01 ^{ns}	-0.02 ^{ns}	-0.03 ^{ns}	-0.03 ^{ns}	0.15**	0.18*	0.16**	0.19***			
Ko	0.83***	0.21***	0.19***	0.05*	0.07*	0.07**	0.04 ^{ns}	0.06*	-0.01 ^{ns}	-0.02 ^{ns}	-0.002 ^{ns}	0.10*		
Ku	0.85***	0.29***	0.24***	0.08*	0.10*	0.09*	0.09 ^{ns}	0.06 ^{ns}	-0.02 ^{ns}	-0.02 ^{ns}	-0.007 ^{ns}	0.18*	-0.03 ^{ns}	
Bk	0.58***	0.29**	0.30**	0.30***	0.29**	0.37***	0.28**	0.34***	0.39***	0.30***	0.45***	0.22*	0.35***	0.27*

Table 3 Hierarchical analysis of molecular variance (AMOVA) of mtDNA COI sequences in *Tridacna crocea* from the Indo-Malay Archipelago. For abbreviations of sample sites see Table 1. * = 0.05 ≥ p ≥ 0.01, ** = 0.01 > p ≥ 0.001, *** = p < 0.001; ns = not significant.

Region groupings	Φ_{CT}	% variance among groups
(Pa) (PS, Ka) (KK, Sa, Ma, Sp, Bi, Se, Ke, Lu, TI, Ko, Ku) (Bk)	**0.50*** **	**50.80**
(Pa) (PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku) (Bk)	0.37***	36.87
(Pa, PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku) (Bk)	0.17^{ns}	17.26
(Pa, PS, Ka, KK) (Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku) (Bk)	0.14^{ns}	13.83
(Pa) (PS, Ka, KK) (Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku) (Bk)	0.35***	35.51
(Pa) (PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI) (Ko, Ku) (Bk)	0.34***	33.98
(Pa) (PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Bk) (Ko, Ku)	0.22**	22.37
(Pa) (PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku, Bk)	0.26**	26.46
(Pa, PS, Ka) (KK, Sa, Ma, Sp, Bi) (Se, Ke, Lu, TI, Ko, Ku, Bk)	0.06*	5.98

Figure 1

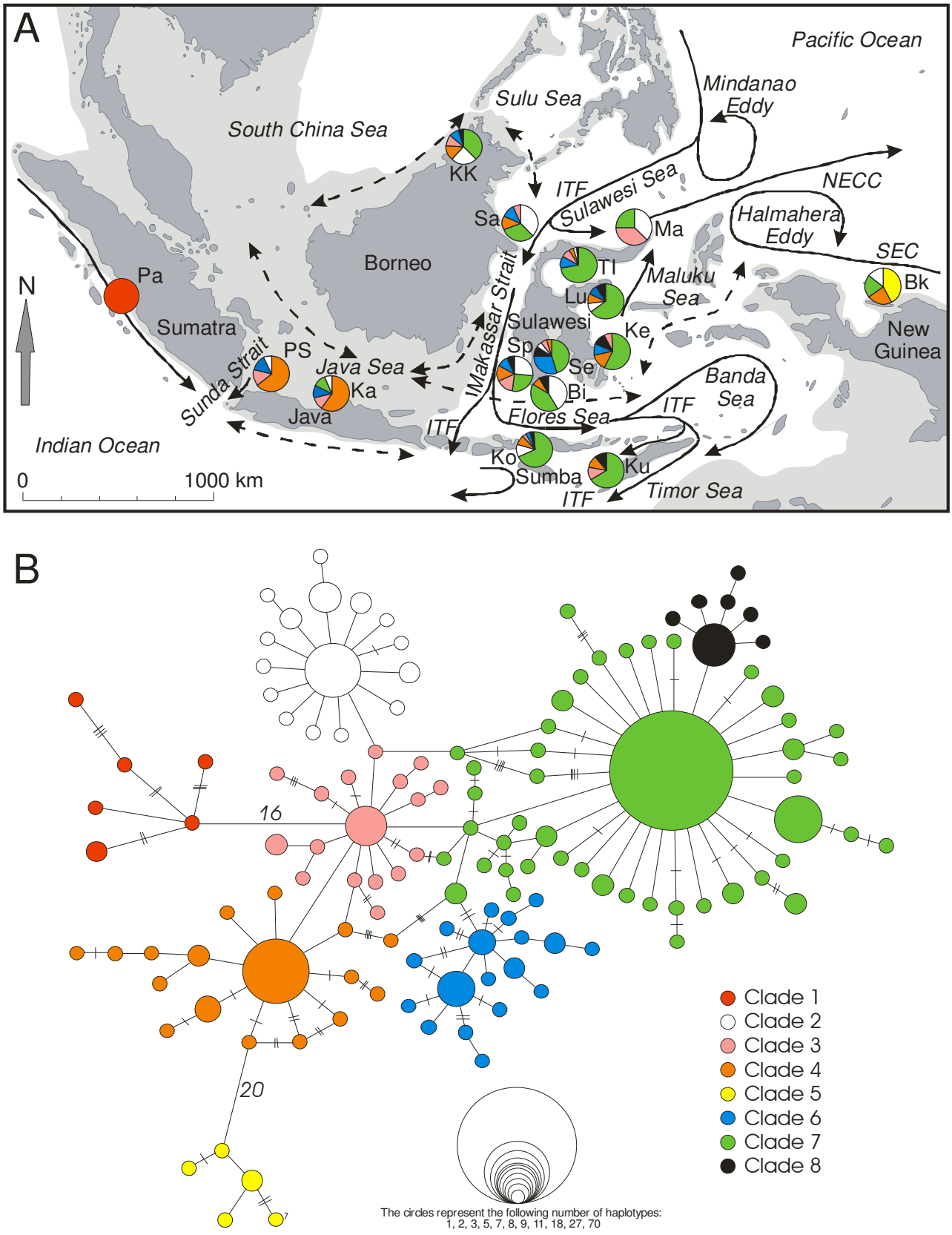
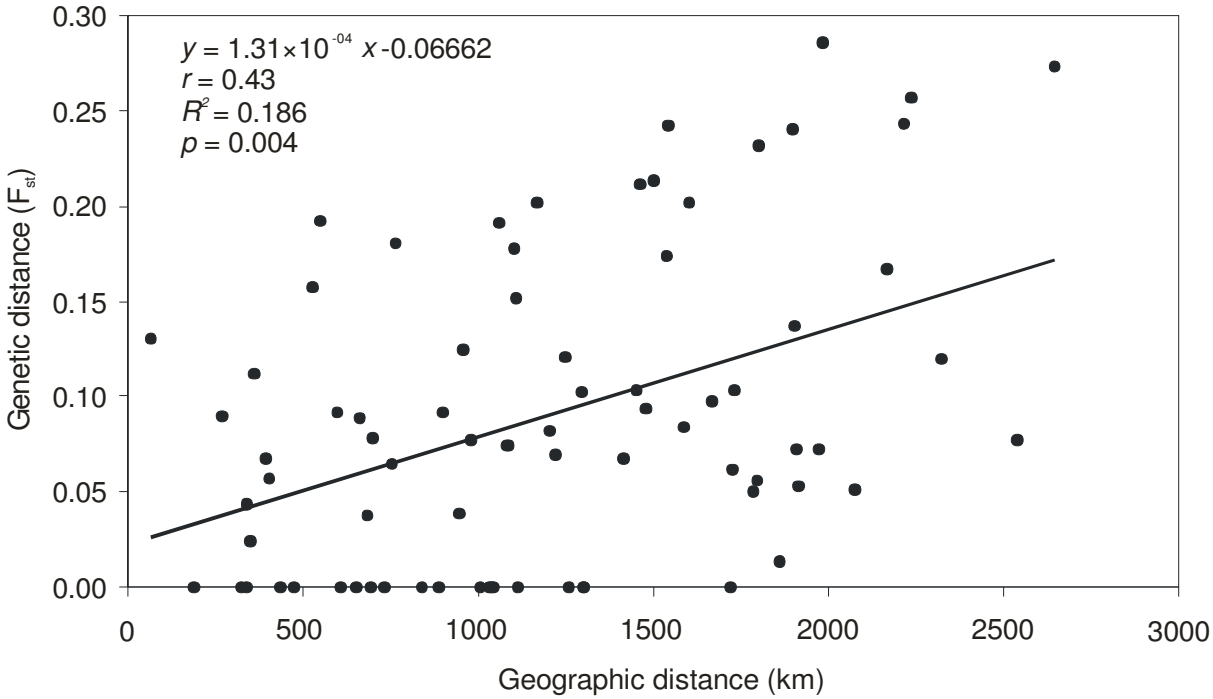

A
Pacific Ocean
Mindanao Eddy
Sulu Sea
South China Sea
KK
ITF
Sulawesi Sea
NECC
Halmahera Eddy
Sa
Ma
SEC
Borneo
Tl
Bk
Maluku Sea
Lu
Pa
N
Sumatra
Sulawesi
Ke
New Guinea
Makassar Strait
PS
Sp
Se
Java Sea
Sunda Strait
Ka
Bi
Banda Sea
ITF
Java
Flores Sea
ITF
Indian Ocean
Ko
Ku
Sumba
Timor Sea
ITF
0
1000 km
B
16
20
Clade 1
Clade 2
Clade 3
Clade 4
Clade 5
Clade 6
Clade 7
Clade 8
The circles represent the following number of haplotypes:
1, 2, 3, 5, 7, 8, 9, 11, 18, 27, 70

Figure 2


y = 1.31×10^-04 x -0.06662
r = 0.43
R² = 0.186
p = 0.004
Genetic distance (Fst)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
0
500
1000
1500
2000
2500
3000
Geographic distance (km)

Restricted gene flow between populations of the giant clam *Tridacna maxima* (Tridacnidae, Bivalvia) in the Indo-West Pacific

Agus Nuryanto, Marc Kochzius

Manuscript in preparation for *Heredity*

Introduction

Tridacna maxima, also called great or rugose clam, is the geographically most wide spread species of giant clam and is distributed from the Red Sea and Western Indian Ocean across the Indo-Malay Archipelago to the Tuamotu Archipelago in the central Pacific. This species grows up to 35 cm shell length and is characterised by bright and various colour pattern. Dispersal is only mediated by a short planktonic larvae duration of about nine days (Lucas 1988).

According to CITES data, international trade with non-captive bred giant clams increased from about 40,000 specimens in 1993 to about 100,000 in 2001 (Wabnitz *et al*. 2003). Such as other giant clam species, wild stocks of *T. maxima* in the Indo-Malay Archipelago are heavily exploited for food, shells, and aquarium trade (Lucas 1994, Wells 1997). Even though giant clams are fully protected in Indonesia and collection, sale and export of wild specimens is banned, enforcement of this legislation is poor (Wells 1997). Clam meat has been consumed extensively by the coastal population in Indonesia and shells were used for different purposes, such as basins, ornaments, and production of tiles (Pasaribu 1988, Wells 1997). Levels of exploitation in the Western Indian Ocean (Wells 1997) and Red Sea are not known.

Due to their bright colouration pattern, *T. maxima* is a popular species in the aquarium trade. Main importers for giant clams in the marine ornamental trade are the European Union and the United States of America with about 48,000 and

45,000 specimens in 2001, respectively (Wabnitz *et al*. 2003). All tridacnid species are listed in Appendix II of CITES, allowing international trade only with appropriate export permits. *T. maxima* are classified in the IUCN Red List of Threatened Animals as Lower Risk: Conservation Dependent.

Even though the Indo-Malay Archipelago, which is located in the coral triangle between Sumatra, the Philippines, and New Guinea, is the centre of marine biodiversity (Briggs 1999), genetic diversity and structure of marine animals is not studied intensively. However, such studies are important to understand evolutionary and ecological processes that lead to the extremely high biodiversity in this region. Additionally, the inference of gene flow and connectivity among populations is important for the conservation of species. Marine protected areas (MPAs) are considered to be a helpful tool to prevent overexploitation in order to ensure the sustainable use of living marine resources (Palumbi 2001). It is proposed that MPAs should be arranged in a network and that the spatial distribution should match the dispersal capabilities of the species to be protected (Palumbi 2003).

Currently, studies on the genetic population structure and gene flow of marine organisms within the Indo-Malay Archipelago investigated only a few invertebrates, such as the giant tiger prawn (Sugama *et al*. 2002), and mantis shrimps (Barber *et al*. 2002, Barber *et al.* 2006), as well as fishes, such as scad mackerels (Arnaud *et al*. 1999; Perrin and Borsa 2001, Borsa 2003), an anemone fish (Nelson *et al*. 2000), job fish (Ovenden *et al*. 2004), and seahorses (Lourie *et al.* 2005). Of these species, only seahorses and mantis shrimps are well covered, with many populations sampled across the archipelago. Currently, only one study investigated the genetic population structure of a giant clam (*T. crocea*) across the Indo-Malay Archipelago (Nuryanto & Kochzius, unpublished data), even though several studies deal with population genetics of giant clams

already (Ayala *et al*. 1973, Campbell *et al.* 1975, Benzie & Williams 1992a, b, Macaranas *et al*. 1992, Benzie & Williams 1995, Benzie & Williams, 1997, Kittiwattanawong 1997, Yu *et al*. 2000, Kittiwattanawong *et al.* 2001, Laurent *et al*. 2002, Juinio-Meñez *et al*. 2003).

Distribution of genetic variation and connectivity among populations can be inferred with molecular markers, such as the cytochrome c oxidase I gene (COI). Studies on the population genetics of bivalves have shown that COI is a suitable marker for such studies (e.g. Luttikhuizen *et al*. 2003, Shefer *et al*. 2004).

Here, we study the genetic structure of *T. maxima* populations in the Indo-West Pacific (Red Sea and Indo-Malay Archipelago) based on COI sequences to infer gene flow (connectivity) among populations. Special emphasis will be given to populations in the Indo-Malay Archipelago to provide baseline data for conservation.

Material and Methods

Sampling

Mantle tissue of *T. maxima* was collected during field trips in 2004 and 2005 at 14 sample sites across the Indo-Malay Archipelago and in the Red Sea (Fig. 1; Table 1). In order to avoid collecting the complete specimens, small pieces of mantle tissue were cut off under water. Tissue samples were preserved in 96% of ethanol and later on stored at 4°C.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated using the Chelex® method, following the protocol from Walsh *et al*. (1991). In this study we use the cytochrome c oxidase I (COI) gene as a molecular marker. A fragment of this gene was amplified using a pair of tridacnid-specific primers: COI-Tricro-Frwd 5'-GGG TGA TAA TTC GAA CAG AA-3' and COI-Tricro-Rev 5'-TAG TTA AAG CCC CAG CTA AA-3' (reverse). The PCR reaction was carried out in a total volume of 50 µl. PCR reactions

contained 1 µl DNA template, 10 mM Tris-HCl (pH 9), 50 mM KCl, 2 mM of $MgCl_2$, 0.2 µM of each primer, 0.2 mM of each dNTP, and 1 unit Taq polymerase. Thermal cycles were as follows: one cycle at 94 °C for 3 minutes, followed by 35 cycles of 1 minute at 94 °C, 1.5 minutes at 50 °C, and 1 minute at 72 °C. Final extension was performed at 72 °C for five minutes. The PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) or Merchery Nagel PCR purification kit following the protocol from the manufacturer. Both strands were sequenced using the DyeDeoxy terminator chemistry (PE Biosystem, Foster City, USA) and an automated sequencer (ABI prism 310; Applied Biosystems, Foster City, U.S.A.).

DNA sequence analysis and gene diversity

All sequences were edited using the programme Sequence Navigator (ver.1.0.1; Applied Biosystems) and checked manually by eye. The sequences were checked for orthology to sequences of *Tridacna crocea* (Nuryanto & Kochzius, unpublished data). The DNA sequences were translated to amino acid sequences with the programme Squint (http://www.cebl.auckland.ac.nz/index.php) in order to exclude mistakes in sequencing and to verify if a functional mitochondrial DNA sequence was obtained and not a nuclear pseudogene.

A multiple sequences alignment was obtained with ClustalW (Thompson *et al.* 1994) as implemented in the software Bioedit (ver. 7.0.4.1; Hall 1999). Genetic diversity was estimated using haplotype diversity *h* (Nei 1987) and nucleotide diversity π (Nei and Jin 1989). Both genetic diversity indices were carried out by using the software Alrlequin (ver. 2.0; Schneider *et al*. 2000)

Genetic population structure analysis

Significance of genetic structure among populations was estimated with Analysis of Molecular Variance (AMOVA; Excoffier *et al*. 1992) and pairwise genetic

different Φ_{ST} values. Both analyses were performed with the programme Arlequin (ver. 2.0; Schneider *et al*. 2000) with a gamma shape parameter of 0.22 and the Tamura Nei model taken into account. The gamma shape parameter and evolutionary model were obtained with the programme Modeltest (ver. 3.7; Posada and Crandall 1998). A haplotype network showing the relationships among haplotypes was created using the programme Arlequin (ver. 2.0; Schneider, *et al*., 2000) and drawn by hand.

The null hypothesis of neutral evolution of the COI gene was tested using Tajima's D test (Tajima 1989) and Fu's Fs test (Fu 1997) with the programme Arlequin (ver. 2.0; Schneider *et al*. 2000). In order to obtain a reliable result the test was carried out with 10,000 randomisations. Negative value of Tajima's D value either indicates population hitchhiking, a bottle neck or population expansion. The sudden population expansion model was also tested based on 10,000 permutations as implemented in Arlequin (Rogers 1995).

Isolation by distance analysis was conducted to test for a correlation between genetic and geographic distances. The correlations were estimated using Reduced Major Axis (RMA) regression analysis. Correlation of genetic distances and geographic distances between each pair of populations was calculated using the program isolation by distance web service (IBD WS 3.13) with 10,000 permutations to test for significance (Jensen *et al.* 2005; http://ibdws.sdsu.edu). Geographic distances were measured with an electronic world atlas, distances representing the shortest path between two populations by sea.

Results

A length of 484 bp of a COI gene fragment was obtained from 211 individuals of *T. maxima* collected at 14 localities across the Indo-Malay Archipelago and in the Red Sea. From these 211 individuals a total number of 117 haplotypes were

obtained. Among 484 bp a total of 116 (23.97%) polymorphic sites were observed with 122 mutations.

Genetic diversity within population showed high levels of haplotype diversity, except for the populations from Karimunjava, Spermonde and Komodo. In general, low levels of nucleotide diversity below one percent were observed, except for the population from Biak. Haplotype diversity ranges from 0.59 in the population from Karimunjava to 1.00 in Bira, nucleotide diversity from 0.24% in Komodo to 4.51% in Biak (Table 1). Overall haplotype diversity (*h*) was 0.94 and nucleotide diversity (π) was 3.2 %.

Tajima's *D* test (*D* = -2.35; *p* = 0.08) and Fu's *Fs* test (*Fs* = -24.07; *p* = 0.002) rejected the null hypothesis of neutral evolution of the COI marker. However, this could indicate population expansion, which is supported by the mismatch distribution analysis (Figure 2) and Rogers' test of sudden population expansion (sum of square deviations, SSD: *p* = 0.58; raggedness index *r*: *p* = 0.77; Rogers 1995). Therefore, the marker can be assumed to be neutral.

Genetic population structure

Evolutionary relationships among 117 haplotypes from *T. maxima* are presented in the haplotype network (Figure 3), showing four different clades. Clade 1 consisted of haplotype from Sulawesi, Borneo, Komodo, Kupang and Biak. Clade 2 consists of haplotype from the Java Sea, Padang and Borneo; whereas Clade 3 and 4 are exclusively include haplotypes from the Red Sea and Biak, respectively. The most common haplotype was found in a frequency of 22.3% and was unique for the eastern region of Indonesia (from Makassar Strait eastward to Papua). The second most common haplotype occurred in 9.5% of the individuals and was unique for the populations from Borneo westward to the Java Sea and Padang.

The significance of genetic structure among *T. maxima* populations was verified by AMOVA and pairwise Φ_{st} values. These analyses considered a gamma shape parameter of 0.22 and the Tamura Nei substitution model, showing a highly significant genetic structure among *T. maxima* populations in the Indo-West Pacific (Indo-Malay Archipelago and the Red Sea; $\Phi_{st} = 0.74$, $p < 0.001$) and across the Indo-Malay Archipelago ($\Phi_{st} = 0.72$, $p < 0.001$). Pairwise Φ_{st}-values among sample sites showed a complexity pattern of significant differences and homogeneity. Most of pairwise comparison reveal restricted gene flow (58.2%), the remaining indicate panmixing (Table 3). Significant Φ_{st} –values range from 0.06 to 0.91. The populations from the Red Sea, Padang, Pulau Seribu, Karimunjava and Biak are the four most divergent populations, whereas populations around Sulawesi show panmixing.

A positive correlations between genetic and geographic distance ($r = 0.61$, $p < 0.001$; Figure 4) was found. In this analysis we excluded the genetically different populations from the Red Sea. Isolation by distance was only observed among the population from Padang; Pulau Seribu, Spermonde, Togean Islands and Biak, whereas the populations under the impact of ITF (Makassar Strait) and eastern coast of Sulawesi (including Komodo and Kupang) showing panmixing and no revealed isolation by distances.

Discussion

Genetic diversity

The molecular marker used in this study shows a high level of polymorphism and is sufficient to reveal restricted gene flow between populations of the giant clam *T. maxima*. Levels of genetic diversity are comparable with other study on marine invertebrates (e.g. Arnaud *et al*. 2000, Barber *et al*. 2002, Luttikhuizen *et al*. 2003, Shefer *et al*. 2004, Duran *et al*. 2004). High levels of haplotype diversity and low levels of nucleotide diversity in COI are common in marine

invertebrates (Benzie et al. 2003, Duran et al. 2004, Shefer et al. 2004). The low level of genetic diversity in the population from Karimunjava is difficult to explain since Pulau Seribu showed a high degree of genetic variation. There are two potential factors leading to the low genetic diversity in the Java Sea. On the one hand, low genetic diversity could be due to a founder effect, which is caused by the re-colonisation of the Sunda Shelf with the rising sea level after the last glacial. On the other hand, the low genetic diversity in the Java Sea could be due to over-exploitation, because giant clams in the Java Sea have been heavily harvested material in the so-called "teraso" tile industry (Pasaribu 1988, Wells 1997). However, since the populations from Pulau Seribu showed high level genetic diversity, the low degree of genetic diversity seems to be not due to re-colonisation of the Sunda shelf. Therefore, it seems more likely that over-exploitation in Karimunjava is the reason for the low genetic diversity. However, populations of the giant clam *T. crocea* (Nuryanto & Kochzius, unpublished data) showed a low genetic populations structure in both populations.

Demographic history

Neutrality tests (Tajima's *D* and Fu's *Fs*) rejected the null hypothesis of neutral evolution of the utilised molecular marker. However, these tests can not differentiate between selection and demographic changes of populations. Therefore, Rogers' test to examine the null hypothesis of sudden demographic expansion was performed (Rogers & Harpending 1992, Rogers 1995). The data show a unimodal distribution of pairwise differences which indicates a recent population expansion. It is therefore suggested that a sudden demographic expansion seems to be the reason for the rejection of null hypothesis of neutral evolution. Such a population expansion can be explained by the availability of new habitats after the rise of the sea level at the end of the last glacial, which enabled growth of the reduced populations (Fauvelot *et al.* 2003). The same

signal of a sudden population expansion was found in *T. crocea* from the Indo-Malay Archipelago (Nuryanto and Kochzius, unpublished data).

Genetic population structure and connectivity

T. maxima shows a very strong genetic population structure across the Indo-Malay Archipelago, with a Φ_{st}-value of 0.72. The Φ_{st}-value even reaches 0.74 when the Red Sea population is included. The observed Φ_{st}-value in the Indo-Malay Archipelago is very high compared to other studies on giant clams. A study on *T. crocea* in the same region revealed a Φ_{st}-value of 0.28 (Nuryanto and Kochzius, unpublished data). Comparisons to other studies are difficult, because allozymes have been used as a genetic marker. A study on *T. crocea* in the Philippines showed a F_{ST}-value of 0.066 (Juinio-Meñez *et al.* 2003), and F_{ST}-values for other giant clams (*T. derasa*, *T. gigas*, and *T. maxima*) ranged from 0 to 0.156 in the Western Pacific (Benzie & Williams 1992a, b, Macaranas *et al.* 1992, Benzie & Williams 1995, Benzie & Willimas 1997). A number of studies on *T. maxima* populations have shown different pattern of genetic structure. For example, a high degree of protein polymorphism was reported in *T. maxima* at Eniwetok Atoll (Ayala *et al*. 1973). High genetic variability was also reported for population from the Great Barrier Reef (Campbell *et al*. 1975). Panmixia in *T. maxima* populations was observed along the Great Barrier Reef (Benzie & Williams 1992a), but significant differences in allozyme allele frequencies could be detected among different archipelagos in the West and Central Pacific (Benzie and Williams 1997). Furthermore, a significant genetic structure was reported among *T. maxima* populations North and South of Phuket in the Andaman Sea (Kittiwattanawong 1997). In addition, significant genetic structure was observed in *T. maxima* populations from different archipelagos in French Polynesia, showing isolation-by-distance (Laurent *et al*. 2002).

According to the pronounced genetic differences, the sample sites can be divided into five groups from West to East: (1) Red Sea, (2) Padang (Eastern Indian Ocean), (3) Java Sea, (4) Sulawesi (including Sangalaki, Komodo, and Kupang), and (5) Biak (Western Pacific). Such differentiation from West to East in the Indo-Malay Archipelago was also found in *T. crocea,* but these populations also showed a separation between the western and eastern coats of Sulawesi (Nuryanto & Kochzius, unpublished data), which could not be found in *T. maxima*. A similar pattern was also found in other studies, but it is difficult to make a comparison due to the differences in the geographic resolution of sampling. The seahorse *Hippocampus spinosissimus* showed a genetic break between the Indian Ocean and Java Sea as well as South China Sea, whereas *H. trimaculatus* exhibited a genetic differentiation in the Eastern Java Sea (Lourie *et al.* 2005). Three species of mantis shrimp showed a genetic separation between Papua and the islands west of it (Barber *et al.* 2006). An additional genetic differentiation between northern and southern populations was observed for the mantis shrimp *Haptosquilla pulchella* (Barber *et al.* 2002), but such a pattern was not revealed for *T. maxima*.

A separation between populations of invertebrates between the Red Sea and Indian Ocean was also found in on the crown-of-thorns starfish *Acanthaster planci* (Benzie *et al.* 2000) and the mud crab *Scylla serrata* (Fratini and Vannini 2002), but could not be detected in the lionfish *Pterois miles* (Kochzius & Blohm 2005).

A genetic break between Indian and Pacific Ocean, as revealed in *T. maxima*, was also detected in several other populations, such as starfish (Williams & Benzie 1998), mantis shrimp (Barber *et al*. 2002, Barber *et al.* 2006), and fishes (Chenoweth *et al*. 1998, Nelson *et al*. 2000). This separation can also be shown for sibling species, e.g. in lionfish (Kochzius *et al.* 2003), butterfly fish (McMillan

& Palumbi 1995), and starfish (Williams 2000). The differentiation between populations of the Indian and Pacific Ocean could be due to low sea level during the glacial. In those times, the Java Sea on the Sunda shelf between Java and Borneo was dry land. This created a geographic barrier between the two ocean basins and limited genetic exchange between populations in the Indian and Pacific Ocean (McManus 1985, Voris, 2000).

The populations from the Java Sea (Pulau Seribu and Karimunjava) were not statistically different, indicating high gene flow and panmixing (Table 3). This could be due to seasonal changing currents in the Java Sea (Figure 1) which connect these populations that are more than 400 km apart. Furthermore, the pelagic larval durations of nine days (Lucas 1988) seem to be sufficient for *T. crocea* to travel such distance mediated by currents.

Populations from northern Borneo (Sangalaki), around Sulawesi, Komodo, and Kupang (Timor) do not show significant Φ_{st}-values, indicating panmixing. Populations along the Indonesian throughflow (ITF) (Sangalaki, Manado, Spermonde, Bira, Komodo, and Kupang) are well connected by the strong and steady current. A similar pattern could also be observed for the giant clam *T. crocea* (Nuryanto & Kochzius, unpublished data), but in this species isolation-by-distance is present along the ITF, which could not be detected in *T. maxima*. Additionally, *T. crocea* showed a significant genetic differentiation between the western and eastern coast of Sulawesi, which is not present in *T. maxima*. Such different pattern of connectivity in the two giant clam species around Sulawesi might be due to differences in larval ecology and dispersal capabilities. A study on a mantis shrimp revealed a strong genetic isolation of the population from Tomini Bay (Barber *et al.* 2002), which is not present in *T. maxima* and *T. crocea*. The two giant clam species rather exhibit a similar pattern as a scad

mackerel which showed not genetic heterogeneity between populations in Tomini Bay and the Molluccas (Arnaud *et al.* 1999).

This complex genetic population structure, characterised by panmixing between some sites and restricted gene flow between others can be attributed to prevailing current regimes and the geological history of the Indo-Malay Archipelago. The major observed genetic differentiation between the Indian Ocean and Western Pacific is most probably due to historical isolation by sea level changes, whereas current oceanographic conditions facilitate connectivity along the ITF and separation between other regions. These factors cause vicariance between populations, which can lead to allopatric speciation, suggesting that the Indo-Malay Archipelago is a centre-of-evolutionary-radiation (Briggs 1999).

Implication for conservation in the Indo-Malay Archipelago

It is strongly recommended to manage Indonesia's marine fisheries (Mous *et al*. 2005) and marine protected areas (MPAs) are considered to be a helpful tool to prevent overexploitation. Even though 51 MPAs with an area of about 58,000 km^2 in Indonesia include coral reefs (Spalding et al. 2001), these MPAs cover only about 1 % of the total marine area of the Indonesian Archipelago.

The strong genetic population structure of *T. maxima* across the Indo-Malay Archipelago has important implications for the conservation of this species. The four defined groups in the Indo-Malay Archipelago (Padang; Java Sea; Sulawesi, including Sangalaki, Komodo and Kupang; Biak) should be managed separately, because exchange between the regions is limited. The small scale genetic differentiations in some areas suggest limited larval dispersal, which should be considered in the spatial arrangement of MPAs.

In order to draw a generalised picture of connectivity among populations in the Indo-Malay Archipelago for the design of a functioning MPA network further studies on other invertebrates and fishes are needed.

References

Arnaud S, Monteforte M, Galtier N, Bonhomme F, Blanc, F (2000). Population structure and genetic variability of pearl oyster *Pinctada mazatlanica* along Pacific coast from Mexico to Panama. *Conserv Genet* **1**: 300–307.

Arnaud S, Bonhomme F, Borsa P (1999). Mitochondrial DNA analysis of the genetic relationships among populations of scads mackerel (*Decapterus macarellus*, *D. macrosoma*, and *D. russelli*) in South-East Asia. *Mar Biol* **135**: 699-707.

Ayala FJ, Hedgecock D, Zumwalt GS, Valentine JW (1973). Genetic variation in *Tridacna maxima*, an ecological analog of some unsuccessful evolutionary lineages. *Evolution* **27**: 177-191.

Barber PH, Erdmann MV, Palumbi SR (2006). Comparative phylogeography of three codistributed stomatopods: origins and timing of regional lineage diversification in the coral triangle. *Evolution* **60**: 1825-1839.

Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2002). Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes and consequences. *Mol Ecol* **11**: 659-674.

Benzie JAH, Williams ST (1992a). Genetic structure of giant clam (*Tridacna maxima*) populations from reefs in the Western Coral Sea. *Coral Reefs* **11**: 135-141.

Benzie JAH, Williams ST (1992b). No genetic differentiation of giant clam (*Tridacna gigas*) populations in the Great Barrier Reef, Australia. *Mar Biol* **112**: 1-5.

Benzie JAH, Williams ST (1995). Gene flow among giant clam (*Tridacna gigas*) populations in Pacific does not parallel ocean circulation. *Mar Biol* **123**: 781-787.

Benzie JAH, Williams ST (1997). Genetic structure of giant clam (*Tridacna maxima*) populations in the west Pacific is not consistent with dispersal by present-day ocean currents. *Evolution* **51**: 768-783.

Benzie JAH, Smith C, Sugama K (2003). Mitochondrial DNA reveals genetic differentiation between Australian and Indonesian pearl oyster *Pinctada maxima* (Jameson 1901) populations. *J Shellfish Res* **22**: 781-787.

Benzie JAH, De Vantier L, Turak E, Ballment E, Peplow L (2000). Relative genetic isolation of Red Sea populations of crown-of-thorns starfish (*Acanthaster planci*). Abstracts of the 9th International Coral Reef Symposium, Bali, Indonesia, p 290.

Briggs JC (1999). Coincident biogeographic patterns: Indo-West Pacific Ocean. *Evolution* **53**: 326-335).

Campbell CA, Valentine JW, Ayala FJ (1975) High genetic variability in a population of *Tridacna maxima* from the Great Barrier Reef. *Mar Biol* **33:** 341-345.

Chenoweth SF, Hughes JM, Keenan CP, Lavery S (1998). When oceans meet: a teleost shows secondary integration at an Indian-Pacific interface. *Proc R Soc Lond B Biol Sci* **265**: 415-420.

Duran S, Palacin C, Becerro MA, Turon X, Giribet G (2004). Genetic diversity and population genetic of the commercially harvested sea urchin *Paracentrotus lividus* (Echinodermata, Echinoidea). *Mol Ecol* **13**: 3317–3328.

Excoffier L, Smouse PE, Quattro JM (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**: 479-491.

Fauvelot C, Bernardi G, Planes S (2003). Reduction in the mitochondrial DNA diversity of coral reef fish provides evidence of population bottlenecks resulting from Holocene sea-level change. *Evolution* **57**: 1571-1583.

Fratini S, Vannini M (2002). Genetic differentiation in the mud crab *Scylla serrata* (Decapoda: Portunidae) within the Indian Ocean. *J Exp Mar Biol Ecol* **272**: 103-116.

Fu FX (1997. Statistical test of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915-925.

Gordon AL (2005). Oceanography of the Indonesian seas and their throughflow. *Oceanography* **18**: 14-27.

Hall TA (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp*, **41**: 95-98.

Jensen JL, Bohonak AJ, Kelley ST (2005). Isolation by distance, web service. BMC Genet **6**; 13.

Juinio-Meñez MA, Magsino RM, Ravago-Gotanco R, Yu ET (2003). Genetic structure of *Linckia laevigata* and *Tridacna crocea* populations in the Palawan shelf and shoal reefs. *Mar Biol* **142**: 717-726.

Kittiwattanawong K (1997) Genetic structure of giant clam, *Tridacna maxima* in the Andaman Sea, Thailand. *Spec Publ Phuket Mar Biol Cent* **17**: 109–114.

Kochzius M, Söller R, Khalaf MA, Blohm D (2003). Molecular phylogeny of the lionfish genera *Dendrochirus* and *Pterois* (Scorpaenidae, Pteroinae) based on mitochondrial DNA sequences. *Mol Phylogenet Evol* **28**: 396-403.

Kochzius M, Blohm D (2005). Genetic population structure of the lionfish *Pterois miles* (Scorpaenidae, Pteroinae) in the Gulf of Aqaba and northern Red Sea. *Gene* **347**: 295-301.

Laurent V, Planes S, Salvat B (2002). High variability of genetic pattern in giant clam (*Tridacna maxima*) populations within French Polynesia. *Biol J Linn Soc*, **77**: 221-231.

Lucas JS (1988). Giant clams: description, distribution and life history. In: Copland JW, Lucas JS (eds) Giant Clams in Asia and the Pacific, Australian Centre for International Agricultural Research: Canberra. pp 21-32.

Lucas JS (1994). The biology, exploitation and mariculture of giant clams (Tridacnidae). *Rev Fish Sci* **2**: 181-223.

Luttikhuizen PC, Drent J, Baker AJ (2003). Disjunct distribution of highly diverged mitochondrial lineage clade and population subdivision in a marine bivalve with pelagic larval dispersal. *Mol Ecol* **12**; 2215-2229.

Macaranas JM, Ablan CA, Pante MJR, Benzie JAH, Williams STH (1992). Genetic structure of giant clam (*Tridacna derasa*) populations from reefs in the Indo-Pacific. *Mar Biol* **113**: 231-238.

McManus JW (1985). Marine speciation, tectonics and sea-level changes in Southeast Asia. *Proceedings of the Fifth International Coral Reef Congress, Tahiti* **4**: 133–138.

McMillan WO, Palumbi SR (1995). Concordant evolutionary patterns among Indo-West Pacific butterfly fishes. *Proc R Soc Lond B Biol Sci* **260**: 229-236.

Mous PJ, Pet JS, Arifin Z, Djohani R, Erdmann MV, Halim A, Knight H, Pet-Soede L, Wiadnya G (2005). Policy needs to improve marine capture fisheries management and to define a role for marine protected areas in Indonesia. *Fish Manag Ecol* **12**: 259-268.

Nei M (1987). *Molecular evolutionary genetics*. Columbia University Press: New York.

Nei M, Jin L (1989). Variances of the average numbers of nucleotide substitutions within and between populations. *Mol Biol Evol* ***6***: 290-300.

Nelson JS, Hoddell RJ, Chou LM, Chan WK, Phang VPE (2000). Phylogeographic structure of false clownfish, *Amphiprion ocellaris*, explained by sea level changes on the Sunda shelf. *Mar Biol* **137**: 727-736.

Palumbi SR (2001). The ecology of marine protected areas. In: Bertness MD, Gaines SM, Hixon ME (eds) *Marine Community Ecology*. Sinauer Associates: Sunderland. pp 509-530.

Palumbi SR (2003). Population genetics, demographic connectivity, and the design of marine reserves. *Ecol Appl*, **13**: S146-S158.

Pasaribu BP (1988). Status of giant clams in Indonesia. In: Copland JW, Lucas JS (eds) *Giant Clams in Asia and the Pacific*, Australian Centre for International Agricultural Research: Canberra. pp 44-46.

Posada D, Crandall KA (1998). Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**: 817-818.

Rogers AR (1995). Genetic evidence for Pleistocene population expansion. *Evolution* **49**: 608-615.

Rogers AL, Harpending H (1992). Population growth makes in the distribution of pairwise genetics differences. *Mol Biol Evol* **9**: 552-569.

Schneider S, Roessli D, Excoffier L (2000). Arlequin, version 2.000. University of Geneva: Geneva.

Shefer S, Abelson A, Mokady O, Geffen E (2004). Red to Mediterranean Sea bioinvasion: natural drift through the Suez Canal, or anthropogenic transport? *Mol Ecol* **12**: 2333-2343.

Tajima F (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* **123**: 585-595.

Thompson JG, Higgins DG, Gibson TJ (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignments through sequence weighting, position specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**: 4673–4680.

Voris HK (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time duration. *J Biogeogr* **27**: 1153–1167.

Wabnitz C, Taylor M, Green E, Razak T (2003). From ocean to aquarium. UNEP-WCMC: Cambridge, UK.

Walsh PS, Metzger DA, Higushi R (1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *BioTechniques* **10**: 506-513.

Wells S (1997). Giant clams: status, trade and mariculture, and the role of CITES management. IUCN: Gland, Switzerland, and Cambridge, UK.

Williams ST (2000). Species boundaries in the starfish genus *Linckia*. *Mar Biol* **136**: 137-148.

Wyrtki K (1961). Physical oceanography of the Southeast Asian waters. University of California: La Jolla, USA.

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Figure Legends

Figure 1: Map of the Indo-Malay Archipelago showing samples sites (for abbreviations see Table 1) as well as oceanographic pattern with dominant (solid lines) and seasonally changing (dashed lines) currents (Wyrtki 1961, Gordon 2005; *ITF*: Indonesian throughflow). Pleistocene maximum sea level low stand is indicated by the light grey area (Voris 2000).

Figure 2 Network of 117 mitochondrial cytochrome c oxidase I haplotypes from 211 individuals of *Tridacna maxima* collected in the Indo-Malay Archipelago and the Red Sea. Connecting lines between circles represent one mutational step. The hatches and numbers indicate additional mutational steps. The size of the circles is proportional to haplotype frequency.

Figure 3 Mismatch distribution of mitochondrial cytochrome c oxidase I sequences of *Tridacna maxima*. The curves represent the frequency distribution of pairwise differences.

Figure 4 Relationship between genetic versus geographic distances in *Tridacna maxima* using Reduced Mayor Axis (RMA) regression with only considering specimens from Red Sea, Padang, Pulau Seribu, Spermonde, Togen Islands and Biak.

Table1 Sample sites, number of sequences (n), number of halpotypes (Nhp), haplotype diversity (*h*) and nucleotide diversity (π) of mitochondrial cytochrome c oxidase I sequences in *Tridacna maxima* from the Indo-Malay Archipelago and the Red Sea.

Code	Population	n	Nhp	*h*	π (%)
RS	Red Sea	13	10	0.95 ± 0.051	0.59 ± 0.37
Pa	Padang	15	10	0.92 ± 0.053	0.53 ± 0.34
PS	Pulau Seribu	12	9	0.91 ± 0.080	0.32 ± 0.23
Ka	Karimunjava	20	8	0.59 ± 0.130	0.60 ± 0.37
Sa	Sangalaki	7	5	0.86 ± 0.137	0.84 ± 0.54
Sp	Spermonde	21	10	0.69 ± 0.115	0.56 ± 0.34
Bi	Bira	10	10	1.00 ± 0.045	0.76 ± 0.48
Se	Sembilan	12	8	0.85 ± 0.104	0.46 ± 0.31
Lu	Luwuk	16	10	0.90 ± 0.062	0.45 ± 0.30
TI	Togian Islands	21	18	0.99 ± 0.019	0.86 ± 0.50
Ma	Manado	22	15	0.90 ± 0.057	0.43 ± 0.28
Ko	Komodo	12	7	0.77 ± 0.128	0.24 ± 0.19
Ku	Kupang	9	7	0.89 ± 0.081	0.81 ± 0.49
Bk	Biak	16	14	0.98 ± 0.028	4.51 ± 2.35

Table 2 Hierarchical analysis of molecular variance (AMOVA) of of mitochondrial cytochrome c oxidase I sequences in *Tridacna maxima* from the Indo-Malay Archipelago and the Red Sea. For abbreviations of sample sites see Table 1. * $0.05 \geq p \geq 0.01$, ** $0.01 > p \geq 0.001$, *** $p < 0.001$.

Groupings	Φ_{CT}	% variance among groups
(RS) (Pa, PS, Ka) (Sa, Sp, Bi, Se, Lu, TI, Ma, Ko, Ku) (Bk)	**0.838*** **	**83.76**
(RS) (Pa) (PS, Ka) (Sa, Sp, Bi, Se, Lu, TI, Ma, Ko, Ku) (Bk)	0.833***	83.27
(RS) (Pa) (PS, Ka) (Sa, Sp, Bi, Ma, Ko, Ku) (Se, Lu, TI,) (Bk)	0.788***	78,82
(Pa, PS, Ka) (Sa, Sp, Bi, Se, Lu, TI, Ma, Ko, Ku) (Bk)	**0.834*** **	**83.36**
(Pa) (PS, Ka) (Sa, Sp, Bi, Ma, Se, Lu, TI, Ko, Ku) (Bk)	0.827***	82.71
(Pa) (PS, Ka) (Sa, Sp, Bi, Ma, Ko, Ku) (Se, Lu, TI,) (Bk)	0.770***	77.01

Table 3 Pairwise Φ_{st}-values between samples site in the Indo-Malay Archipelago and the Red Sea. The values are based on mitochondrial cytochrome c oxidase I sequences of *Tridacna maxima*. For abbreviations of sample sites see Table 1. * $p \leq 0.05$.

	RS	Pa	PS	Ka	Sa	Sp	Bi	Se	Lu	TI	Ma	Ko	Ku
Pa	0.89*												
PS	0.91*	0.06*											
Ka	0.88*	0.03	-0.01										
Sa	0.85*	0.82*	0.86*	0.80*									
Sp	0.88*	0.82*	0.85*	0.80*	0.02								
Bi	0.86*	0.82*	0.85*	0.80*	-0.06	0.02							
Se	0.89*	0.85*	0.89*	0.83*	0.001	0.01	-0.01						
Lu	0.89*	0.86*	0.88*	0.83*	0.03	0.03	0.01	0.01					
TI	0.85*	0.81*	0.82*	0.79*	-0.01	0.03	-0.01	-0.001	-0.01				
Ma	0.89*	0.86*	0.88*	0.84*	0.03	0.02	0.01	0.02	-0.01	-0.01			
Ko	0.91*	0.87*	0.91*	0.84*	0.01	-0.01	-0.01	-0.02	-0.03	-0.03	-0.03		
Ku	0.85*	0.81*	0.84*	0.79*	-0.05	0.04	-0.05	0.01	0.04	0.02	0.04	0.02	
Bk	0.76*	0.79*	0.78*	0.80*	0.66*	0.76*	0.69*	0.72*	0.74*	0.74*	0.77*	0.73*	0.72*

Figure 1

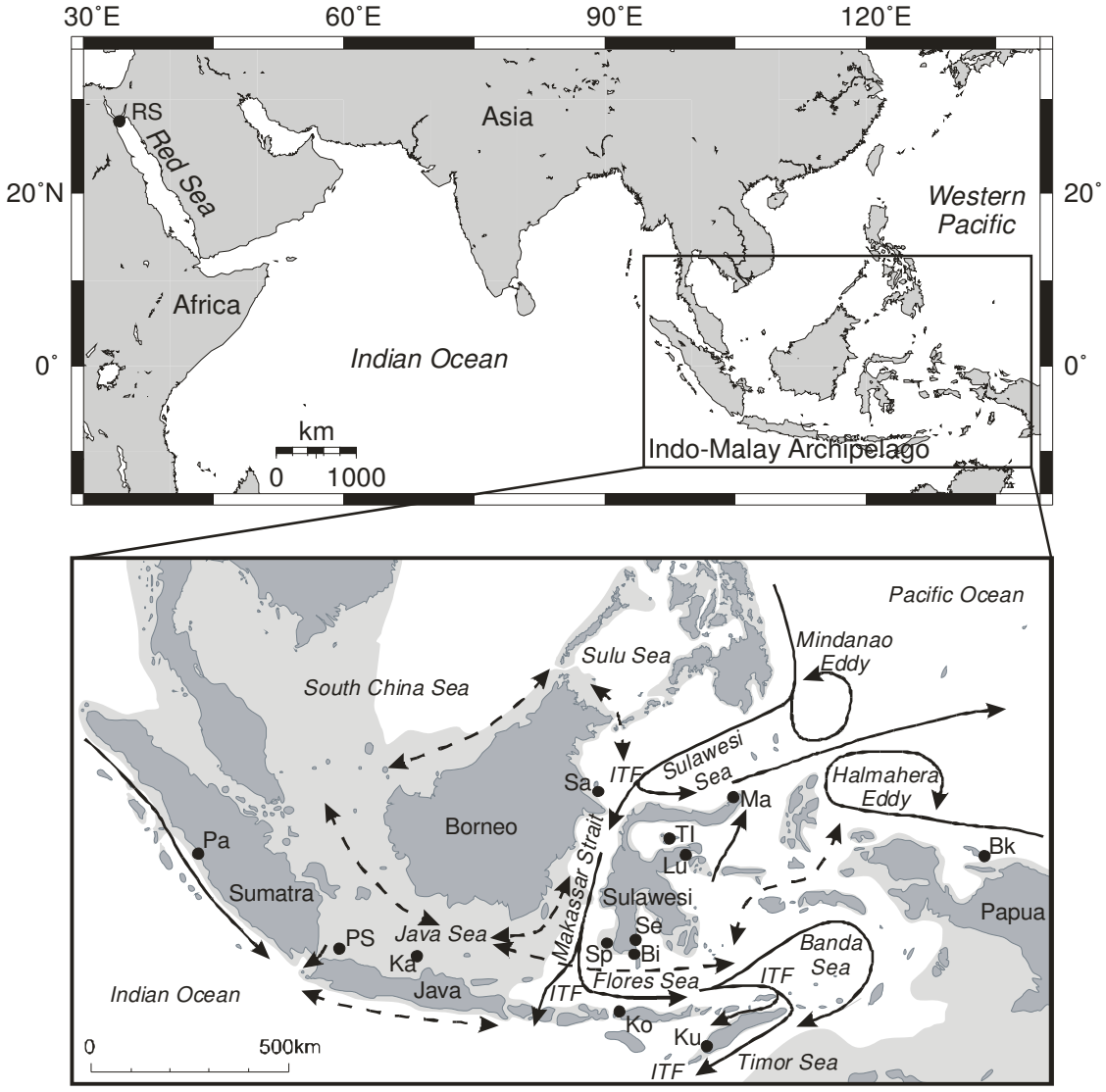


Figure 2

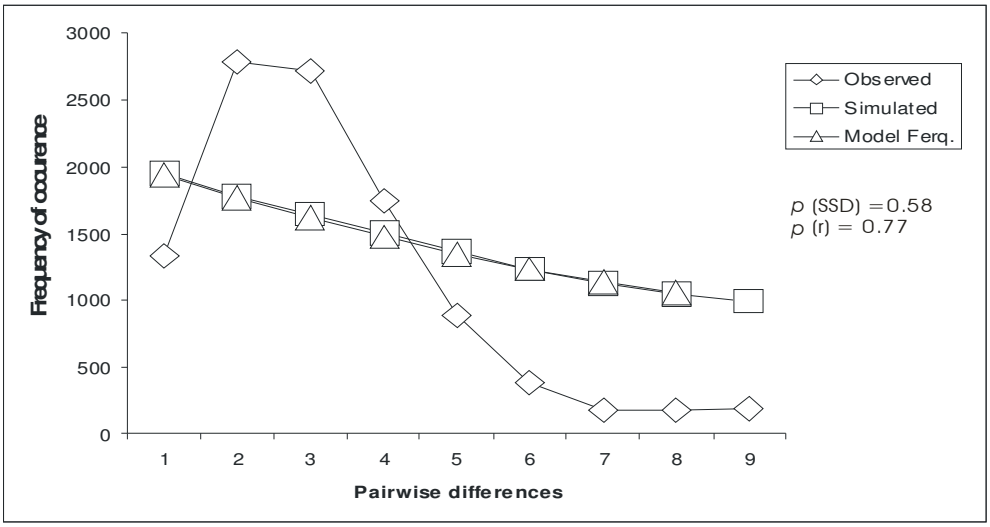


Figure 3

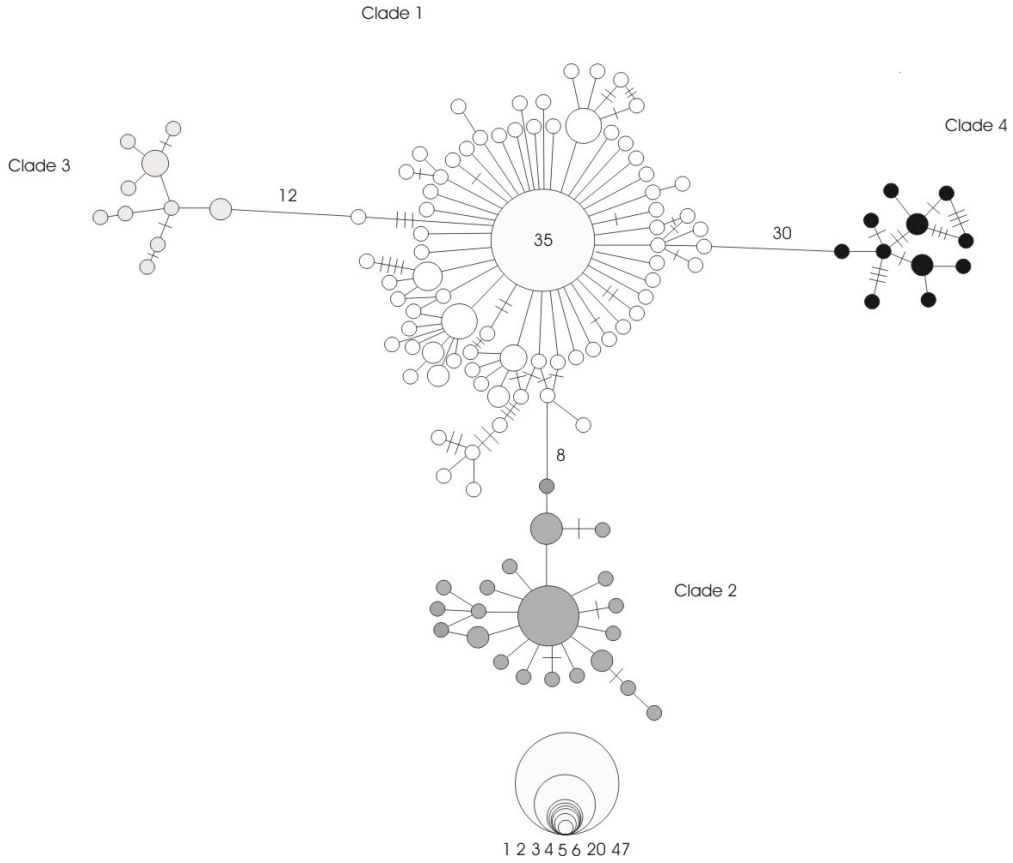
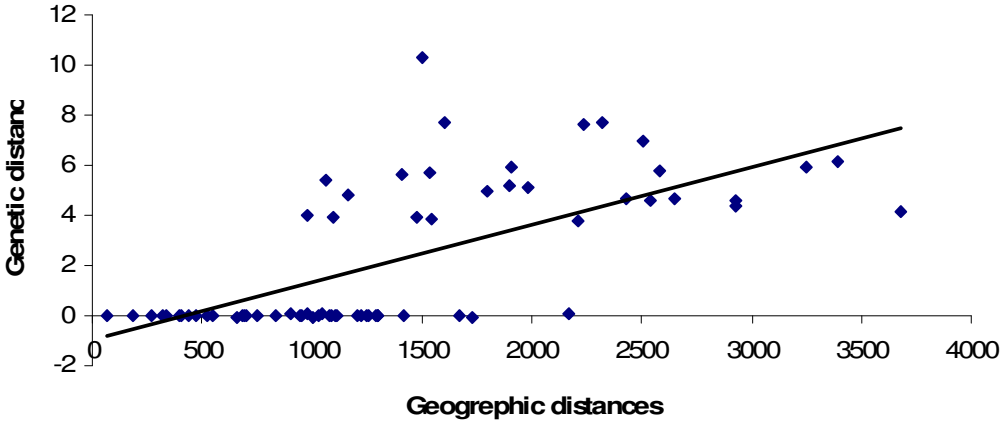


Figure 4


12
10
8
6
4
2
0
-2
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500
1000
1500
2000
2500
3000
3500
4000
Genetic distanc
Geogrephic distances

Congruence of genetic population structure in the blue starfish *Linckia laevigata* and its parasite *Thyca crystallina* in the central Indo-Malay Archipelago?

Kochzius M, Hauschild J, Kirchhoff S, Meyer-Wachsmuth I, Seidel C, Mester P

Manuscript in preparation for *Coral Reefs*

Introduction

The blue starfish *Linckia laevigata* lives on shallow coral reefs throughout the Indo-Pacific. It hosts amongst other parasites *Thyca crystallina*, a small, blue and cockle-shaped snail. Adults have a rather small home range and therefore only the larval stage provides the potential for long distance dispersal[1]. During the lowered sea level in the last ice age, the connectivity between populations in the Indian and Pacific Ocean was restricted. Therefore, a marine Wallace line between the Indian and Pacific Ocean is proposed, located along an axis in the Flores and Java Sea from West to East[2]. Assuming an increase of the Indonesian throughflow (ITF) from North to South after the last glaciation[3], a heterogeneous composition of haplotypes in the central Indo-Malay Archipelago could be supposed. It could be expected that the re-colonisation followed the throughflow.
Here, we investigate if (1) a population structure in *Linckia* laevigata and *Thyca crystallina* can be observed, and if (2) the population structures of both organisms are congruent.

Materials and Methods

Samples from *Linckia laevigata* and *Thyca crystallina* were collected the Indo-Malay Archipelago. We used the mitochondrial cytochrome oxidase 1 gene (COI) as a marker to determine sequence variation in both species. For standard PCR we used the universal COI-Primers from Folmer et al.[4], but had to design new sequencing primers for each of the two species. For the analysis we used sequences of 204 individuals from 23 sample sites with 395 bp length from *L. laevigata* and sequences of 181 individuals from 16 sample

sites with 409 bp length from *T. crystallina*. The data set of *L. laevigata* includes 35 sequences from GenBank published by Williams et al. (2000[7], 2002[3]). We calculated haplotype networks with the software TCS, evaluated the best-fit evolutionary model with the programmes PAUP* and Modeltest, and conducted the population genetic analysis (AMOVA) with the software Arlequin. A Neighbour Joining (NJ) tree was constructed with the software MEGA. Neutrality test Tajima's D and mismatch distribution was conducted with Arlequin. Isolation by distance was analysed with IBD Web Service at http://phage.sdsu.edu/~jensen/.

Results and Discussion

A neutrality test was performed to verify if the COI marker is under selection (Tab. 1). For *L. laevigata* as well as *T. crystallina* Tajima's D is negative (-1.6 and –1.7 respectively) and the *p* values are significant (0.02 and 0.03 respectively), indicating that the marker might be under selection[6]. The test can be influenced by population expansion, bottleneck events or heterogeneity of mutation rates. The analyses of mismatch distribution (p=0.74 and p=0.35 respectively) reveal that the negative D values can be explained by a population expansion. The ratio of the counted nonsynonymous (Dn) and synonymous (Ds) mutations (Dn/Ds=0.14 and 0.16 respectively) suggests that purifying selection is occurring, which decreases deleterious mutations (Dn)[6]. Therefore, we conclude that the COI gene for both species is neutral and can be used as a marker for population genetic approaches.

Table 1: Results of AMOVA, Tajimas's test, mismatch distribution, substitution rates and isolation by distance (IBD) for *L. laevigata* and *T. crystallina*.

Species	**Tajima's Test** a) Tajima's D b) *p* value	**Substitution rate** a) nonsynonymous b) synonymous	**Mismatch distribution** sudden expansion model	**AMOVA** a) FST b) *p* value	**IBD** a) r b) *p* value
Linckia laevigata	a) -1.6 b) 0.02	a) Dn: 9 b) Ds: 63 ◊ Dn/Ds = 0.14	p=0.74	a) 0.09 b) < 0.01	a) 0.6 b) < 0.01
Thyca	a) -1.7	a) Dn: 6	p=0.35	a) 0.02	a) 0.2

crystallina	b) 0.03	b) Ds: 38 ◊ Dn/Ds = 0.16		b) 0.1	b) 0.08

The haplotype network of *L. laevigata* revealed three dominant haplotypes and two clades that are separated by four mutations (Fig. 1a). The proportion of individuals from the two different clades is calculated for each sample site (Fig. 2a). This analysis shows that clade A originates in the Indian Ocean, whereas clade B dominates the Western Pacific. In *T. crystallina* such a strong separation can not be observed (Fig. 2b). The haplotype network shows four dominant haplotypes, but clades are difficult to define. However, the division in two clades (A and B; Fig.1b) is supported by a NJ tree (not shown). The geographical distribution indicates no clear differentiation between the two ocean basins in this area, both clades occur at almost every sample site. The observed genetic structure of two clades could be due to the separation of populations in the Indian and Pacific Ocean during sea level low stands.

a) b)

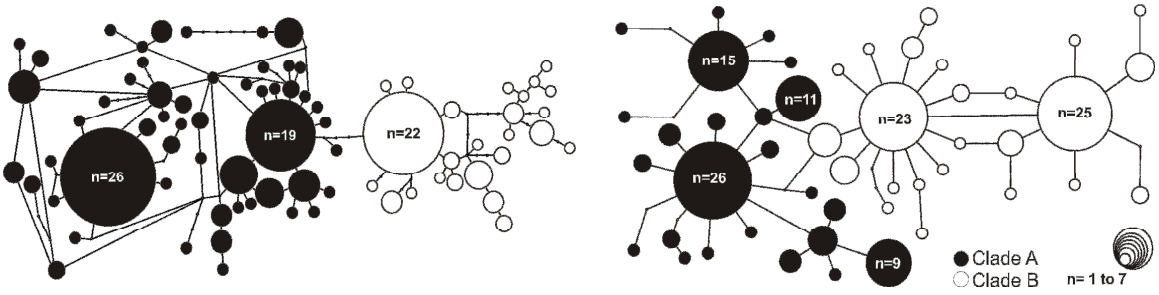
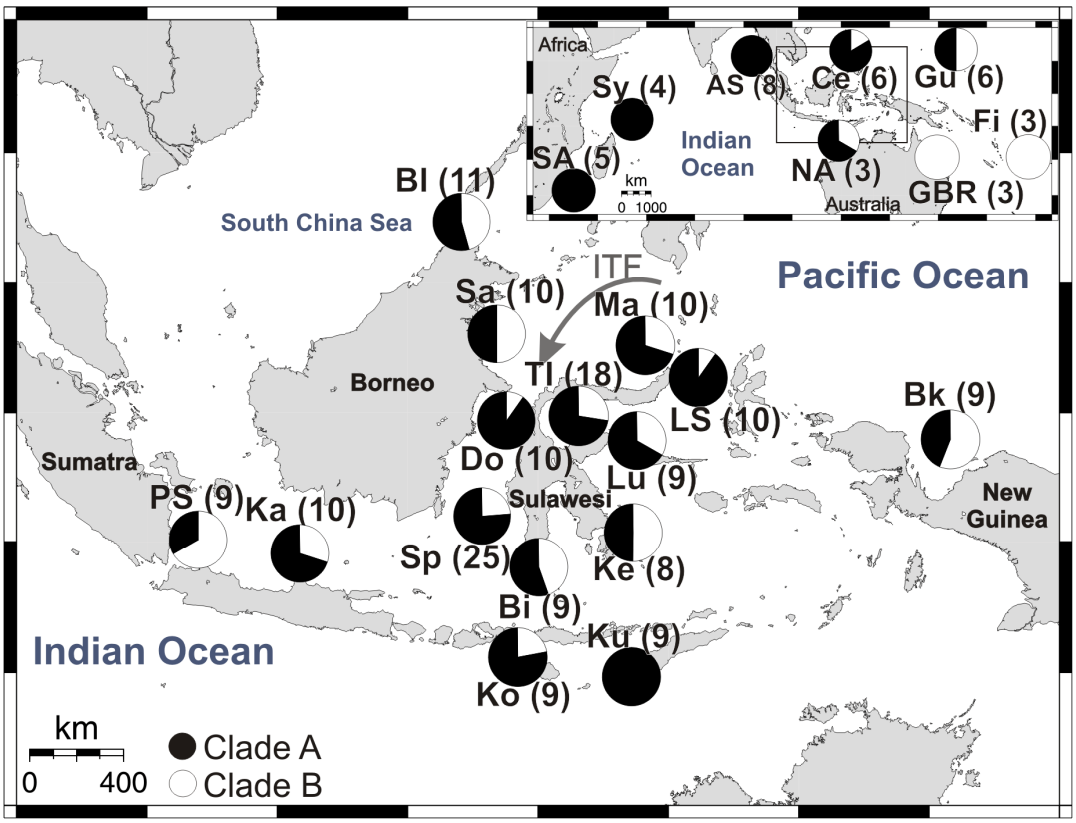


Figure 1: Network of *a)* 79 haplotypes of *Linckia laevigata* and *b)* 47 haplotypes of *Thyca crystallina*. Two clades are indicated in black (A) and white (B). Circles represent haplotypes and their size correlates with abundance.

a)


Africa
Sy (4)
AS (8)
Ce (6)
Gu (6)
Fi (3)
SA (5)
Indian Ocean
NA (3)
GBR (3)
km
0 1000
Australia
Bl (11)
South China Sea
ITF
Pacific Ocean
Sa (10)
Ma (10)
Tl (18)
Borneo
Bk (9)
LS (10)
Sumatra
Do (10)
Lu (9)
PS (9)
Ka (10)
Sulawesi
New Guinea
Sp (25)
Ke (8)
Bi (9)
Ku (9)
Indian Ocean
Ko (9)
km
0 400
Clade A
Clade B

b)

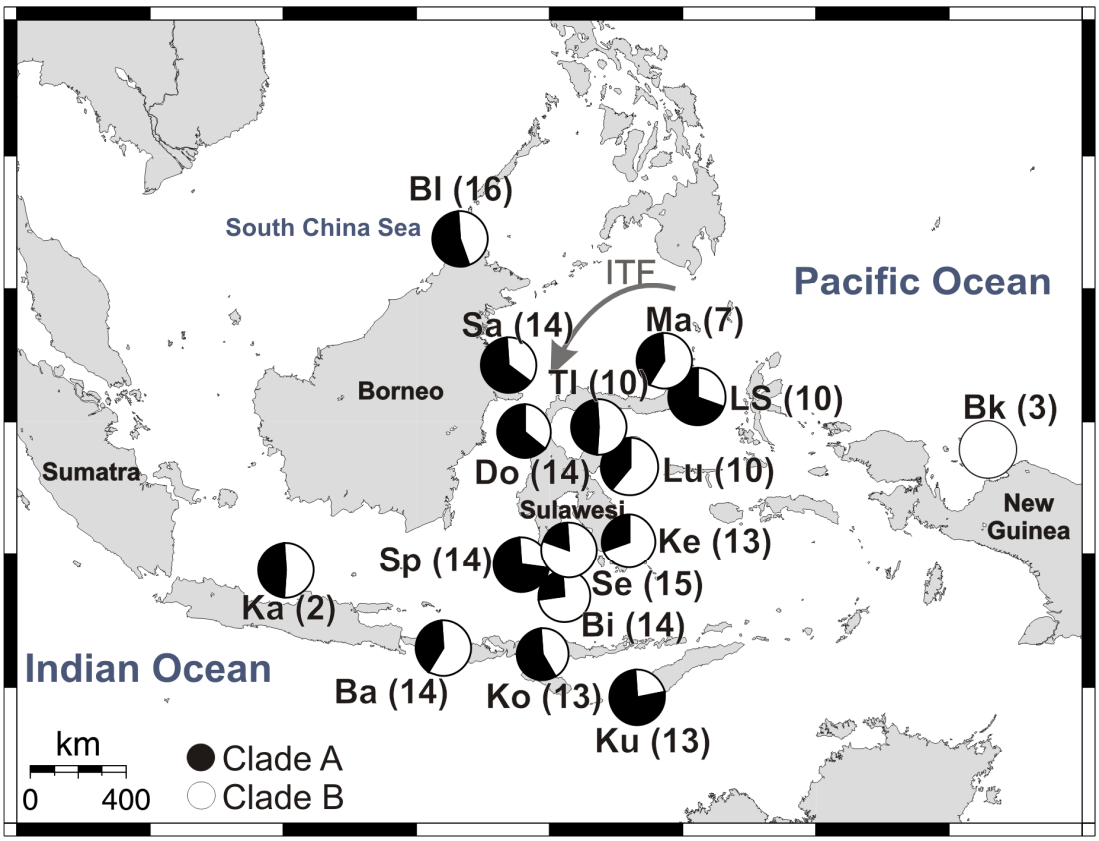


Figure 2: Maps of the Indo-Malay Archipelago located between the Indian and Pacific Ocean. a) *L. laevigata* (in the right upper corner sample sites outside the Indo-Malay Archipelago), b) *T. crystallina*. Pie charts show proportion of individuals from the two different clades for each sample site. (ITF = Indonesian throughflow). Sp: Spermonde; Do: Donggala; Ma: Manado; LS: Lembeh Strait; TI: Togian Islands; Lu: Luwuk; Ke: Kendari; Bi: Bira; Se: Pulau Sembilan (1-9: Sulawesi); Sa: Sangalaki; (Kalimatan); Bk: Biak (Papua); Ka: Karimunjawa (Java Sea); Ba: Padangbai (Bali); Ko: Komodo; Ku: Kupang (Timor); BI: Banggi Islands (Malaysia); PS: Pulau Seribu (Java Sea); SA: South Africa; Sy: Seychelles; AS: Andaman Sea (Thailand); Ce: Cebu (Philippines); Gu: Guam; Fi: Fiji; NA: North-western Australia; GBR: Great Barrier Reef; (n) = number of samples

AMOVA revealed restricted gene flow ($\Phi ST=0.09$; $p<0.01$) in *L. laevigata*, whereas *T. crystallina* did not show a significant genetic differentiation ($\Phi ST=0.02$; $p=0.1$). The isolation by distance analysis documents a significant ($p<0.01$) correlation between the genetic and geographic distance in *L. laevigata*, but *T. crystallina* does not show such a pattern. This might be due to the larger geographic distance between sample sites for *L. laevigata*.

The lack of genetic population structure in *T. crystallina* suggests panmixing, which is probably caused by long distance larval dispersal. Studies on *L. laevigata* also showed panmixing in the Great Barrier Reef, as to be expected due to a rather long planctonic larval phase of 28 days[8]. Considering only sample sites in the Indo-Malay Archipelago, *L. laevigata* also does not show a significant population structure ($\Phi ST=0.03$; $p=0.06$). This congruent results show that host (*L. laevigata*) as well as parasite (*T. crystallina*) have a high larval dispersal capability.

References

[1]Williams ST and Benzie JAH (1997) Mol Ecol 6:559-573

[2]Barber PH, Palumbi SR, Erdmann MV, Moosa MK (2000) Nature 406:692-693

[3]Williams ST, Jara J, Gomez N, Knowlton N (2002) Integr Comp Biol 42:941-952

[4]Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) Mol Mar Biol Biotechnol 3:294–299.

[5]Williams ST (2000) Mar Biol 136:137-148

[6]Skibinski DOF (2000) Hydrobiologia 420:137-152

[7]Willimas ST, Benzie JAH (1993) Mar Biol 117:71-77

Genetic population structure of the Pink Anemonefish, *Amphiprion perideraion* (Pomacentridae), in the Indo-Malay Archipelago

Lemia M.O. Hamid

MSc thesis, ISATEC, University of Bremen

Abstract

The Pink Anemonefish (*Amphiprion perideraion*, Bleeker 1855) is a member of the mainly tropical marine fish family Pomacentridae. It belongs to the genus *Amphiprion* which comprises 26 species of anemonefish. The Pink Anemonefish (*Amphiprion perideraion)* is found in the Indo-Malay Archipelago besides other species. It has a commercial value as a popular aquarium fish traded worldwide. This fish species is characterized by short planktonic larval duration. Most marine fishes disperse as planktonic larvae with a longer duration, and because the distance and direction of dispersal are often unknown, the contributions of different portions of the population to overall population biology are poorly understood. However, dispersal distances and directions have a fundamental effect on gene flow and genetic differentiation within species. Therefore, gene flow in *A. perideraion* was estimated to reveal connectivity among its populations. The primary objective of this study was to investigate the genetic population structure of the Pink Anemonefish, *Amphiprion perideraion*, based on sequence data from the mitochondrial DNA (mtDNA) control region (400 bp) of 247 specimens from 17 populations. The sequences of the mtDNA control region in *A. perideraion* populations showed relatively high polymorphism. A total of 185 haplotypes were obtained. All 17 populations showed a high level of haplotype diversity ranging from 98% to100% and lower nucleotide diversity ranging from 3.7% to 9.01%. Neutrality tests (Tajima's and Fu's) gave non-significant Tajima's D-value ($p > 0.05$) and a significant Fu's F ($p < 0.05$). This result suggests that *A. perideraion* in the Indo-Malay experienced historical bottlenecks in population size or a founder effect followed by rapid population

expansion since the late glacial. This population expansion was confirmed by the non significant difference value of Mismatch Distribution Analysis under the sudden expansion model (p= 0.63). Pairwise Φ_{st} tests showed a genetic differentiation between most populations. The highly significant Φ_{st} value between Karimunjava and the rest of the populations is explained by the history of the population on the Sunda Shelf. These results imply that the population from Karimunjava has undergone isolation at some time and suggest re-colonization from the Indian Ocean during glacial transgression periods due to sea level changes. The Low genetic differentiation between the population of Donggala, Bira and Spermonde compared to the other populations is explained by current effects on larval dispersal. Isolation-by-Distance Analysis showed no correlation between genetic and geographic distances. This pointed to a complex pattern of genetic differentiation and demonstrated limited genetic exchange between populations in this non-migratory fish with short plankton larval duration.

The Analysis of Molecular Variance (AMOVA) of all populations detected highly significant genetic variation (Φ_{st} = 0.096; p< 0.001) and indicates that there is restricted gene flow between populations. The variation within populations was higher than among populations. AMOVA with alternative groupings of populations showed the highest significant difference (Φ_{CT} = 0.125, p<0.0003) between the populations from Karimunjava, Biak, Kendari, Luwuk and the rest of the populations. Therefore, it is concluded that three populations exist in the Indo-Malay Archipelago. In addition, haplotype-networks were constructed and revealed six clades with heterogeneous geographic distributions. However, this still supports the trend of the three groupings. The results suggest that fishery management should consider three different stocks and their management strategies should take into account the genetic differentiation and diversity of this species in the Indo-Malay Archipelago.

1. Introduction

Most marine fishes disperse as planktonic larvae, and because the distance and direction of dispersal are often poorly understood, the contributions of different portions of the population to overall population genetic structure are also poorly understood. At the extremes of their geographic ranges, many species are traditionally thought to settle as benthic juveniles only under favorable conditions, in essence producing a net transport of larvae outward from the center of the range (Cowen 1985, Pringle 1986). Based on other observations, however, it appears that some peripheral populations are in part self-recruiting (Swearer *et al*. 1999, Jones *et al*. 1999, Swearer *et al.* 2002). In many cases behavioral or oceanographic mechanisms may limit large-scale dispersal, even in species that have relatively long pelagic larval durations (Sponaugle *et al*. 2002, Palumbi and Warner 2003). For example, fish larvae may develop the ability to swim against prevailing currents (Leis *et al*. 1996, Fisher *et al*. 2000, Leis and McCormick 2002).

The degree to which such populations are supported by passive larval drift from the center of the range versus self-replenishing is not clear. In examples from populations distributed across archipelagos, population genetic analysis, otolith and mark-recapture techniques have all revealed that populations may often show evidence of population subdivision, in spite of potential long-distance dispersal by pelagic larvae (Schulman and Bermingham 1995, Jones *et al*. 1999, Swearer *et al.* 1999, Planes 2002, Planes *et al*. 1998, Palumbi 2003, Barber *et al*. 2000, Taylor and Hellberg 2003). This is likely for marine organisms whose larvae more probable to be locally retained than to successfully recruit to neighboring populations (Black 1993; Cowen *et al*. 2000; Cowen 2002; Armsworth *et al*. 2001).

Previous studies demonstrate that dispersal makes a measurable contribution to population genetic diversity in the majority of animal species in nature and as a consequence shows relatively low levels of population structure (Bohonak 1999).

For high-dispersal species, the picture is somewhat less clear. For instance, these species often reveal little or no genetic differentiation (Palumbi 1994,

Bohonak 1999). There are also published studies of strong genetic structure among fish species that spend their entire lives as plankton (Darling et al. 2000) or have long planktonic phases (Taylor and Hellberg 2003).

Numerous studies on species in Southeast Asia including works on stomatopod populations (Barber et al. 2002a, 2002b, 2006) , black tiger prawns (Duda and Palumbi 1999), seahorses (Lourie et al. 2005), pearl oysters (Benzie *et al*. 2003) and star fish (Williams *et al*. 1997) have shown that it is possible for genetic differentiation to occur even in the high dispersal species. In spite of these previous mixed results, Palumbi (2003) suggested that even species with a high potential for dispersal may show genetic structure due to limited larval exchange between distant populations. There is less evidence for a similar outcome in fish populations distributed in coral reefs, where genetic differentiation is associated with the duration of their larval stages and their distribution especially in Indo-Malay Archipelago (Woodland 1990). However a fish lacking larval dispersal has also shown genetic differentiation (Doherty *et al*. 1994).

1.1. Molecular Genetic Approach

Molecular tools and observations of mtDNA sequences diversity, especially the control region, provide information for studying gene flow among fish populations (Lee *et al.*1995). To explore the population genetics of the species *A. perideraion* in the Indo-Malay Archipelago, part of control region of the mitochondrial genome (d-loop) was sequenced. The control region includes transcriptional promoters for both strands, the heavy strand replication origin, and the displacement or "D-loop" region (Clayton 1982, Chang and Clayton 1986). Since this is a non-coding region, without the functional constraints associated with protein-coding genes, the control region evolves much faster than the average mitochondrial gene (Brown 1985, lee *et al.* 1995). This high rate of base substitution in the mtDNA control region makes it an ideal choice for addressing population-level genetic questions (Meyer 1991). In addition, because mitochondrial genes are non-recombining and maternally inherited (and therefore represent a

four-fold lower effective population size), they are thought to provide a more sensitive method for detecting population structure than nuclear genes (Birky *et al*. 1989, Avise *et al*. 1987; Parker *et al.* 1998).

The use of highly polymorphic genetic markers such as microsatellites, assayable via the polymerase chain reaction (PCR), has immensely increased our understanding of past and current population genetic processes. However, for most organisms, microsatellite marker's are not available and the development of new ones is time consuming and costly. On the other hand, general primers for certain sequence regions, such as the mitochondrial control region, are often available, or at least quite easily modified to fit a particular organism (Ellegren 2000).

1. 2. Fish species

Amphiprion represent the largest genus of anemone fish with approximately 25 species (Bleeker 1855) ranging all over the Indo-West Pacific region (Fautin and Allen 1992). The faunistic center of *Amphiprion* as well as for most marine taxa is within the Indo-Malayan region which is classically believed to be the center of fish speciation (Ekman 1953). The paleogeography of the Indo-Malayan region has changed dramatically during the Quartenery Period. As sea levels associated with glaciation fell up to 120 m below their present levels, much of the Sunda shelve was terrestrial. Following the formation of a large number of continentally fragmented or volcanic islands, ideal conditions for shallow water anemones and therefore anemone fish were established (Woodlands 1990).The irregularities in the distribution patterns as well as the differences in species diversity denote that many factors play a role in species distribution. For example, the major ocean currents in the region could have different effects (Gordon and Fine, 1996; Godfrey, 1996; Lee *et al*. 2002) and could be a factor in the inter-connectivity of marine areas and aid larval dispersion (Barber *et al*. 2000).

1. 3. General biology of the Pink Anemonefish, *A. perideraion*

The Pink Anemonefish (*Amphiprion perideraion) is* a demersal marine fish found in the tropical water of both the Pacific and the Indian Oceans. It inhabits coastal and offshore coral reefs. *A. perideraion* is typically pink to pinkish orange in colour (Fig.1). Fins are white to transparent. They have a very distinct white dorsal stripe extending from the head to the caudal fin. A second, vertical, stripe is observed between the head and the rest of the body. *A. perideraion* has 9-10 well-developed dorsal spines and 2 anal spines. They have 16-17 dorsal soft rays and 12-13 anal soft rays. Likewise, they have highly developed pharyngeal teeth and a premaxilla with an ascending process, resulting in very effective suction feeding. The fish can reach a maximum length of 10 cm (Lieske and Myers 1994; Fautin and Allen 1992).



Fig.1: *A. perideraion* is typically pink to pinkish orange with distinct white dorsal and vertical Stripes (the picture was taken by Marc Kochzius).

It feeds mainly on phytoplankton but zooplankton and zoobenthos are occasionally in their diet. The population of *A. perideraion* consists of adult,

subadult and juvenile groups. The subadult and juvenile groups are able to mature histologically and change to female should they be largest among conspecific members. Akihisa (2000) suggested that *A. perideraion* in both heterospecific and conspecific groups adopt a mating strategy that involves waiting for vacated breeding posts because of their low mobility and low host density. Recently *A. perideraion* has been identified as a promising candidate for ornamental fish trade and aquaculture (Wabnitz *et al*. 2003) and studies have been carried out on its captive breeding. The reasons to consider *A. periderion* as a valuable culturable species are high growth rate and long lifespan (Fautin and Allen 1992). Species similar to *A. perideraion* include *A. nigripes, A. leucokranos, A. akallopisos* and *A. sandaracinos*. The first two can be distinguished by a black belly, characteristics of the pelvic area and anal fins. The remaining two species lack the white head bar present in *A. perideraion* (Allen 1975; Boyer 2005; Fautin and Allen 1992; Lieske and Myers 1994).

1.3.1. Distribution and Habitat

A. perideraion range includes the Gulf of Thailand, Cocos Islands and Christmas Island in the eastern Indian Ocean. In the Indo-Australian Archipelago, they are found from Samoa and Tongo, north to the Ryukyu Islands, Fiji, and Micronesia, extending southward to the Great Barrier Reef and New Caledonia (Allen 1975; Fautin and Allen 1992; Randall et al 2003) (www.fisbase.org).

These fish are found in lagoons and seaward reefs (Fautin and Allen 1992; Myers, 1991). They are non-migratory living in marine water with depths up to 38 meter. These fish live in symbiotic relationships with the four species of sea anenomes *Heteractis crispa, Hetaractis magnifica, Macrodactyla doreensis* and *Stichodactyla gigantea*. The number of fish that occupies a single anemone depends on the size of the host and sometimes, the size of the fish as well. However there is usually an adult pair and two to four smaller fish (Kuiter & Tonozuka 2001; Myers 1991; Fautin and Allen 1992; Allen 1975).

1.3.2. Development

Once the larvae of *A. perideraion* hatch, they take on a planktonic form. They are then carried from the natal host anemone and float passively in the water column and may allow moderate gene flow among populations. However, *A. perideraion* larvae display a clear shift in behaviour at the onset of feeding, with a drop in overall complexity of their swimming paths and an increase in their swimming speed (Cougling *et al.* 1992).

At the end of their larval period, *A. perideraion* enter a juvenile stage where they metamorphose. Members of the genus *Amphiprion* occupy a single anemone for their entire life, rarely swimming more than several meters from their host (Fautin (1992a). These groups usually consist of one female, but many include several males. The female is the largest member of the colony and the dominant male is the next largest. The others, while male, are functionally sterile unless one of the two dominant fishes die (Allen 1991; Arvedlund *et al*. 2000; Balon 1990; Boyer 2005; Coughlin *et al*. 1992).

1. 3.3. Reproduction

A. perideraion spawning occurs throughout the year (Fautin and Allen 1992). The unusual aspect of sex reversal in this species is that the change is from male to female, i.e., the fish are male first until the female dies (protandrous). During a year, the pair may produce between 2000 and 4000 eggs.

A. perideraion are monogamous fish where only two of the representatives of a group are actually involved in the mating (Allen 1975; Balon 1990; Thresher 1984). While little research has actually been done on the reproductive mechanisms of *A. perideraion*, the species *A. ocellaris* showed similar reproductive mechanisms (Allen 1991; Balon 1990; Boyer 2005). Male *A. perideraion* protects the developing eggs while they are attached to the substrate for about 6 to 8 days. When the larvae hatch, they leave the host anemone as planktonic organisms (Boyer *et al*. 2004).

1. 3. 4. Behavior

A. perideraion live in small social groups made up of a dominant female and male, and accessory, non-reproductive males. They are sedentary as adults, remaining within several meters of their host anemone. Juvenile fish rub against their host anemone in order to develop immunity to the anemone toxin. After immunity is built up, *A. perideraion* live in the anemone as a source of protection from predators. Anemone fish are active during the day. (Boyer 2005; Coughlin *et al*. 1992; Fautin and Allen, 1992).

1. 3. 5. Food Habits and Predation

A. perideraion use suction feeding and exploit a wide range of phytoplankton (blue-green algae and diatoms), zooplankton and zoobenthos. This makes them omnivorous collecting food from surrounding areas near their host anemones (Arvedlund *et al*. 2000 ; Boyer 2005; Fautin and Allen 1992). *A. perideraion* is preyed on by large, predatory fish, notably groupers (Serranidae). The primary defense used by these fish is their ability to survive within sea anemones. The toxic sting of anemones protects resident fish from predators (Allen 1991; Boyer 2005; Boyer et al. 2004; Fautin and Allen 1992).

A. perideraion live with their host anemone in a symbiotic relationship. The toxic stings of anemones protect anemone fish from predators while anemones obtain food particles in the water column as a result of fish feeding. The fish are obligate symbionts of Actinians, occurring exclusively in the tropical shallow water coral reefs. Anemone species used as hosts are *Heteractis crispa*, *Heteractis magnifica*, Macrodactyla *doreensi*s and *Stichodactyla gigantean* (Boyer et al. 2004; Fautin and Allen 1992). Most often this species lives in obligate symbiosis with the sea anemone *H. magnifica*. The Pink Anemonefishare the most thoroughly studied among coral reef fishes, partly because, they keep well in captivity and partly because they remain in the immediate vicinity of the host anemone (Allen 1975).

1.3.6. Economic Importance and Conservation Status

A. perideraion is a common aquarium fish and is thus of economic value to humans. They are quite appealing to divers and their presence may encourage ecotourism (Myers 1991). There are no known adverse effects of *A. perideraion* on humans. They are considered harmless (Allen, 1991). This species is not listed as endangered or threatened and there are no foreseeable plans to place it on the International Union for Conservation of Nature (IUCN) Red List (Myers 1991; Allen 1991).

1. 4. Marine Ornamental Trade

Tropical marine ornamental organisms (fish, corals and other invertebrates) comprise an increasingly important fishery worldwide. The Indo-Malay Archipelago contains more reefs than any other part of the world (Roberts et al. 2002, Lieske and Myers. 1994), and these reefs provide a wide range of benefits. These benefits include jobs and income created through the collection and export of marine aquarium organisms. Ornamental marine species are collected from the Philippines, Indonesia, the Fiji Islands, Sri Lanka and other countries. There are more than 45 exporting countries around the world. However, the significant proportion of stock that enters the global market comes from the two major suppliers, the Philippines and Indonesia (Wood, 2001). Approximately 85 % of the marine aquarium fish exported to the United States and Europe are captured on the reefs of the Philippines and Indonesia alone. The remaining 15 % comes from many other coral reef areas around the world. A more specific study indicated the aquarium fish trade from Indonesia to be worth US$ 32 million/year (Cesar 1996). Reef fish that are part of the ornamental trade are butterflyfish, anemonefish, damselfish, surgeonfish, Moorish idols, wrasse, angelfish, triggerfish, gobies, blennies, basslets, small groupers, pufferfish, rabbitfish, hawkfish, cardinalfish, boxfish and leatherjackets (Pyle, 1993). The most traded species of Pomacentridea is *A. ocellaris*. Since *A. perideraion* belongs to the ten most traded species, it is heavily exploited for use as aquarium fish and the potential for its overexploitation is high. Therefore, there is a

need for ornamental fisheries management and to address the impacts of anemone fish exploitation on species level. Investigations revealed that fish exported annually from the Philippines and Indonesia, which do not yet have fully operational management strategies for their ornamental fisheries (Wood, 2001), had been captured from the wild using damaging methods for example, cyanide (Rubec 1986, 1988; Barber and Pratt, 1997). Therefore, sustainability of diverse marine ornamental fish has been threatened by the high demand and irresponsible practice of explosive and poison fishing.

1.5. Aim of the Study

1.5.1. The Research Problem

This study aims to investigate the genetic population structure of *A. perideraion* to provide genetic data as a basis for future management plans and conservation efforts for this exploited species. Therefore, gene flow (connectivity) between populations of this species was investigated.

1. 5. 2. Hypothesis

This study has the following null hypothesis (Ho): There is no restriction in gene flow between populations. The alternative hypothesis (H_I) is: There is restriction in gene flow between populations, which leads to population structure.

1. 5. 3. Objective of the Study

The main objectives of this study are:

- Investigation of the genetic population structure of *A. perideraion* in the study area.
- Determine the levels of genetic differentiation.
- Estimation of the gene flow (connectivity) among the populations.
- Provide basic genetic data for developing effective management strategies that ensure sustainability and the preservation of ornamental fishery.

1. 5. 4. Significance of the Study

Molecular genetic tools provide a promising way to define the gene flow between populations (connectivity) and detecting different populations. Estimates of larval dispersal are valuable for providing some guidance to conservation efforts. The findings of this study will increase the knowledge of the genetic population structure of *A. perideraion* in the Indo-Malay Archipelago through the obtained genetic data. Also, the findings are expected to assist future investigations and it will give important information for sustainable management strategies for this species which is used in the ornamental trade. Through comparing levels of connectivity among its populations and between this species and another species, the degree to which *A. perideraion* populations in the Indo-Malay Archipelago is threatened can be evaluated. This provides basis for much needed regionally focused management plans. Moreover, this study will provide genetic data which could then be integrated with a variety of ecological, social and policies-oriented data to form a more comprehensive management plan for one of the world's most diverse and threatened ecosystems.

In order to investigate the genetic variability and population structure of the *A. perideraion*, mtDNA control region sequence data were collected from 17 populations in the study area. These data were used to test the hypotheses. These hypotheses were tested by examining the genetic variability of the sequences and estimating gene flow among all populations. mtDNA control region sequence data, which is known to be highly polymorphic within a species due to the highly mutation rate (Meyer 1993) and thus effective to determine the genetic population structure of a species (Palumbi and Baker 1996) was analysed. Based on those genetic data, population histories, with inferences on the evolutionary rate of mtDNA in *A. perideraion*, are discussed.

2. Materials and Methods

2.1. Study area

The study area is the Indo-Malay Archipelago (Fig.2), located between the Indian and Pacific Ocean (latitude 10 N -15 S and longitude 100E -140 E). It consists of around 17.500 islands and has a coastline of about 81.000 km. It is an area with diverse marine coastal ecosystems, such as mangrove forests and coral reefs, and it comprises the world's highest marine biodiversity (Mora *et al*. 2003). The Indo-Malay Archipelago is also characterised as a typical continental shelf. Its shorelines have undergone important changes in the Pleistocene(Hewitt 2000; Voris 2000) with the shallow seas of the Sunda shelf (Java Sea) and Sahul shelf (Arafura Sea) above present sea level, as well as the emergence of land barriers isolating the South China Sea from the Indian Ocean at its southern limit and from the Sulu Sea eastbound. With many pelagic fish populations, these coastal ecosystems are an important source of livelihood for the local population. This region became nowadays an attractive location for ornamental fish trade due to the growing population, and the need to find income resources.

The oceanography and climate in the Indo-Malay Archipelago is dominated by the Indonesian through flow (ITF). A direct Pacific to Indian Ocean current is found through the Sulawesi Sea and Makassar Strait, which flows southward (see arrows in Fig.2). Currents in the study area (Wyrtky 1961) and shorelines at sea level low stands (Voris 2000) are shown in Fig. (2).

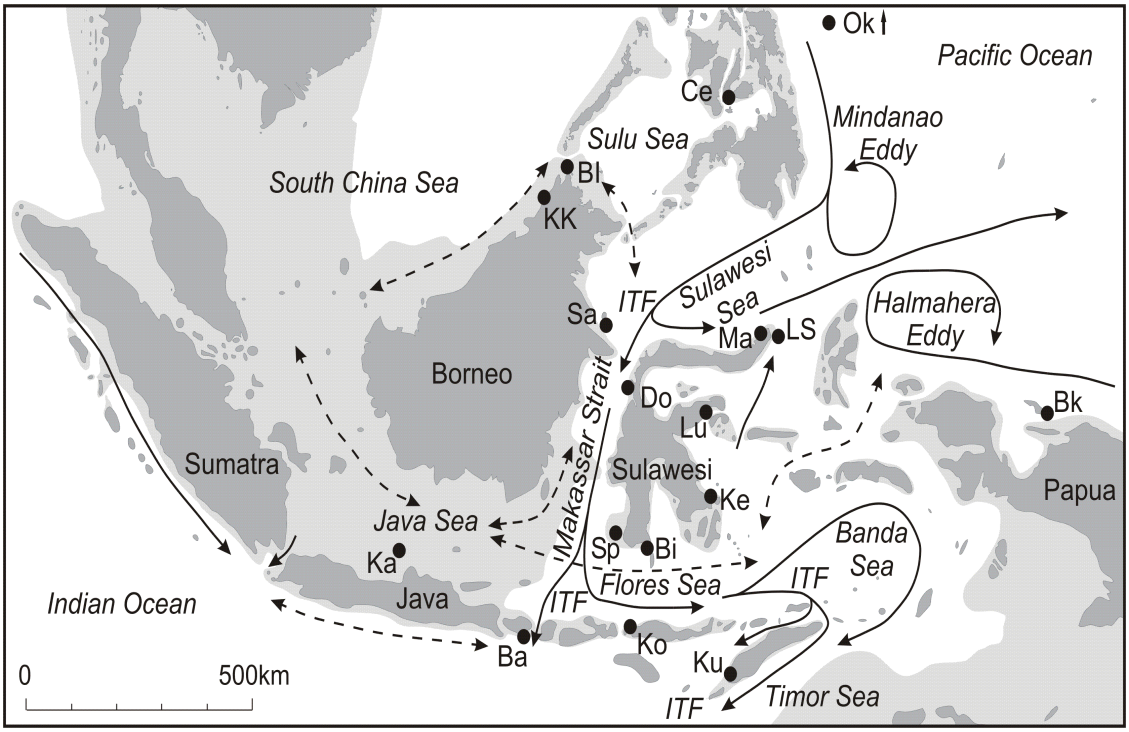


Fig. 2: Study Area in the Indo-Malay Archipelago and sampling sites (for abbreviation see table 1). Currents and Shorelines at sea level low stands in Indo-Malay Archipelago (Voris 2000; Wyrtky 1961).

2. 2. Samples collection and preservation

259 samples of the anemone fish species *Amphiprion periderion* were collected from 17 different locations in the Indo-Malay Archipelago (Fig.2) by Marc Kochzius, Agus Nuryanto and Janne Timm in 2004-2006. Fishes were caught randomly with aquarium hand nets and fin clips of the caudal fins were taken. The different locations and the number of sample are presented in Table (1). Samples were preserved and stored in absolute ethanol and kept at 4°C until the genetic analysis was done.

Table 1: Sampling localities in the Indo- Malay Archipelago of *A. perideraion*, location codes, number of specimens (n) and number of sequences obtained.

locality	***Locality code***	***n***	***sequence***
Banggi Islands, Malaysia	BI	12	11
Donggala, Sulawesi	Do	20	20
Spermonde	Sp	20	20
Komodo, Sulawesi	Ko	10	08
Luwuk, Sulawesi	Lu	14	14
Biak, New Guinea	Bk	22	22
Okinawa, Japan	Ok	12	10
Kendari, Sulawesi	Ke	20	19
Karimunjava, Java Sea	Ka	14	14
Manado, Sulawesi	Ma	21	21
Sangalaki, Kalimantan	Sa	18	17
Kupang, Timor	Ku	16	13
Cebu, Phillippines	Ce	19	19
Bira, Sulawesi	Bi	20	20
Lembeh, Stait, Sulawesi	LS	09	07
Kota Kinabalu, Malaysia	KK	05	05
Bali	Ba	07	07
Total	**17**	**259**	**247**

2. 3. DNA extraction

DNA was extracted from each sample (around 30 mg of tissue) using the peqGOLD Tissue DNA Mini Kits extraction protocol (appendix 1). For homogenization and lysis of each sample a volume of 200 µl TL extraction buffer and 25 µl of OB^{TM} proteinase (20 mg/ ml) were added to the tissues. This was mixed thoroughly by vortexing and then incubated overnight at 55°C in a thermo mixer. Afterwards, DNA was extracted in four successive steps: loading and binding, washing, drying, and elution. First, the

homogenated samples were gently mixed and centrifuged for 1 minute at 9300 rpm with equal volumes (220 µl) of BL-buffer and Ethanol (96%). In the second step, they were centrifuged in order to load the DNA to the HibBind® DNA column. The DNA was then washed twice in 750 µl of washing buffer (diluted with 70% ethanol) and allowed to dry by centrifugation for 5 minutes at 13000 rpm. The dried DNA was gently eluted in 200 µl 70°C warm elution buffer, centrifuged for 2 minutes at 9300 rpm, and then extracted DNA was preserved at -20 °C.

2. 4. Agarose gel electrophoresis of extracted DNA

Agarose gel electrophoresis separates DNA fragments according to their size. An electric current is used to move the DNA molecules across an agarose gel, which is a polysaccharide matrix that functions as a sort of sieve to track the molecules as they are transported by the electric current. The extracted DNA for each sample was run in 1% Agarose gel in 1xTBE buffer (TBE or Tris/Borate/EDTA, is a buffer solution that consists of a mixture of Tris base, boric acid, EDTA, and water). In this gel a mixture of 2 µl DNA and 3µl loading dye (with 6x concentration) was loaded in the gel chamber (Biometra, Goettingen) filled with 1 x TBE buffer (appendix 5).

Volume of 1-2 µl of loading marker was added at each end of the samples row. Then an electric current was passed through the gel containing the mixture for 30 minutes to let the molecules travel through the gel. Then the gel was stained in ethidium bromide for 30 minutes. Finally, the ethidium bromide-stained gel picture was produced under ultraviolet light (see results Fig. 4). The DNA samples are typically run on the same gel with a ladder, which is a sample of DNA where the sizes of the bands are known. So one can compare the unknown fragments to the ladder fragments and determine the approximate size of the unknown DNA bands.

2.5. Polymerase chain reaction (PCR) amplification

For this study a fragment of the mitochondrial control region (d-loop) was amplified by the polymerase chain reaction. The target mtDNA (appendix 2)

was 400 bp long and amplified with the universal primer CR-A (5-TTCCACCTCTAACTCCCAAAGCTAG-3) and universal primer CR-E (5-CCTGAAGTAGGAACCAGATG-3) (Lee et al, 1995). Amplifications were carried out in 25 µl volumes: 2 µl of the extracted DNA, 1 µl of each 10 µM primer CR-A and CR-E, and10 mM dNTPs, 0,1 µl of Taq DNA polymerase (0.5 units), 3 µl of 25 mM MgCl2, 10 x PCR buffer, and purified H2O (see appendix 3). The amplification started with an initial denaturation step at 94°C for 2 minutes, followed by 35 cycles with a profile of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s. The final elongation took place at 72°C for 2 minutes. The amplifications were run in a master cycler® ep S (Eppendorf, Hamburg). Then a gel electrophoresis was done in a 1% Agarose / 1 x TBE-buffer gel in 1 x TBE-buffer for 30 minutes. Afterwards, the gels were stained with 0.5 mg/ml ethidium bromide in 1 x TBE-buffer for 30 minutes and visualised under ultraviolet light to verify the length of the PCR products.

2.6. Purification of DNA

The PCR products which showed the expected length of 400 bp in the gel-electrophoresis were purified with the peqGOLD Cycle-Pure kit (peqLab, Biotechnology, Erlangen) using the procedure given in peqGOLD Cycle-Pure protocol.

2.7. Cycle PCR

PCR products were subsequently subjected to cycle sequencing with the Sanger chain termination method (Sanger, 1977), using the same universal primers. While DNA chains are normally made up of deoxynucleotides (dNTPs), the Sanger method uses Dideoxynucleoside triphosphates (ddNTPs). In this case fluorescent marked ddNTPs (different dye for each nucleotide) were used that were also included next to the usual dNTPs in the Big Dye ready mix (Applied Biosystems). The Dideoxynucleotides (ddNTPs) are missing a hydroxy (OH) group at the 3' position. This position is normally where one nucleotide attaches to another to form a chain. If there is no OH group in the 3' position, the additional nucleotides cannot be added to the

chain, thus interrupting DNA polymerization (elongation). The cycle PCR reaction volume was 10 µl including 3 µl DNA template (purified PCR product), 2 µl Big Dye ready mix, 1µl Primer (CR-A and CR-E separately for each sample), and 4 µl HPLC H2O (See appendix 4). It was run in a master cycler® ep (Eppendorf, Hamburg) and the cycling profiles was 25 cycles, started with 15 s at 94 °C denaturation, 15 sec at 50 °C annealling, and 4 min at 60 °C extension.

2.8. Purification of the cycle PCR products

Prior to sequencing the primers and nucleotides left over from the reaction mix must be removed, therefore the cycle PCR products were purified with the Nucleoseq kit (Macherey-Nagel, Dueren) following the purification protocol.

2.9. Sequencing

The DNA fragments of the cycle PCR were resolved on an automated DNA capillary sequencer. The Sequencing was performed using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Weiterstadt) following the manufacturer's instructions. The detection of the labeled fragments takes place after excitation of the dye by laser. The injection time was 15 seconds for each sample. Therow data was analysed by the program Sequence Analysis (Version 3.4.1).

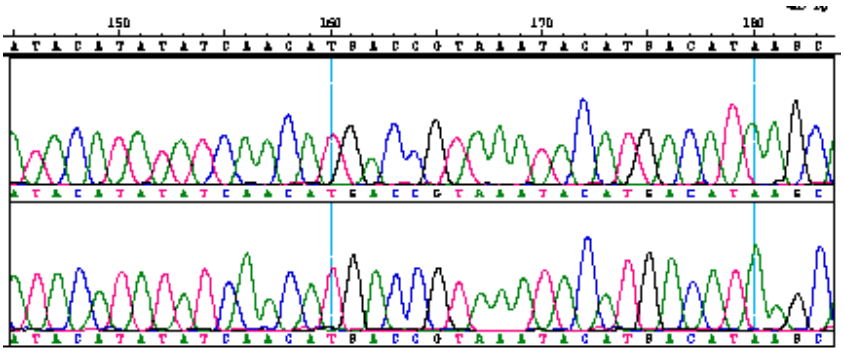


Fig. 3: Example of sequencing read of *A. perideraion* from the Indo-Malay Archipelago. The four bases are detected using different fluorescent labels. These are detected and represented as peaks of different colours, which can then be interpreted to determine the base sequence information.

2.9. Sequence analyses

The DNA sequences obtained were edited with the program SeqMan (Version 4.05, DNASTAR 2001) and by hand in the case of ambiguities (Fig. 3). Then all sequences were aligned using ClustalW as implemented in the software Bioedit (Version7.0, Hall 2004). For population genetics data analysis of the aligned sequences an Analysis of Molecular Variance (AMOVA) was done and the pairwise Φ_{ST} values were calculated with the help of the software Arlequin (Version 4.3, Schneider et al. 2000). Fixation indices (Φ_{ST}) and the corresponding significance values were calculated for the indication of genetic variation and population structure. Tajima's D and Fu's Fs Tests were calculated for confirming selective neutrality of the marker (kimura 1968) also using the Arlequin program. Additionally, Fu's as well as Li's F and D tests were done with the DNASP software (Version 4.10.9). Isolation by distance (IBD) was calculated by the Isolation By Distance Web Service (IBDWS) to test the correlation between genetic and geographic distances (Ngan 2006).

Then the haplotype network for evidence of haplotype clades was done by the software Arlequin (Version 4.3, Schneider *et al*. 2000) and the tree was

drowned by hand with the help of the graphic programmed Coral Draw. For the purpose of this analysis the clade was defined as a group of haplotypes that have fewer mutational steps between haplotypes within the group than between the haplotype groups.

3. Results

3. 1. DNA extraction and PCR

Genomic DNA was successfully extracted from each sample in all 17 populations of *A. perideraion*. The agarose gel picture shows the bands obtained (Fig 4a). Twelve out of the 259 samples were difficult to amplify in the PCR reaction, whereas the remaining showed correct PCR products with a length of 400 base pairs (Fig.4b).

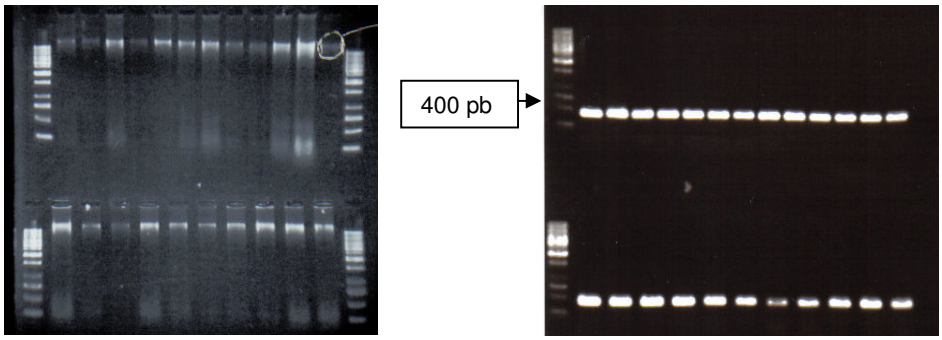


Fig. 4: Ethidium bromide-stained genomic DNA (a) and PCR products (b) after gel electrophoresis. Universal primers (Lee et al 1995) were used to amplify a fragment of the mtDNA control region from 24 different DNA samples. Bands for the samples indicate successful amplification of the DNA by PCR. A DNA ladder containing DNA fragments of defined length (first lane on the left) was added to estimate the sizes of the PCR product.

3. 2. Genetic diversity

All 17 populations of *A. perideraion* showed a high level of haplotype diversity ranging from 98% to100%, and a lower nucleotide diversity ranging

from 3.7% to 9.01% (Table 2). These results imply that the population has experienced expansion.

Table 2: Measures (±S.D.) for haplotype and nucleotide diversities of *A. perideraion* populations in the Indo-Malay Archipelago. Abbreviations for populations are given in Fig (5).

Sample sites	Sample size	No. of haplotypes	Haplotype diversity	Nucleotide diversity (%)
BI	11	11	1.00 ± 0.04	3.67 ± 2.00
Do	20	15	0.96 ± 0.03	4.76 ± 2.45
Sp	20	13	0.95 ± 0.03	4.76 ± 2.45
Ko	8	8	1.00 ± 0.06	4.31 ± 2.40
Lu	14	11	0.97 ± 0.04	5.91 ± 3.10
Bk	22	19	0.97 ± 0.03	7.18 ± 3.60
Ok	10	10	1.00 ± 0.05	3.86 ± 2.10
Ke	19	19	1.00 ± 0.02	7.25 ± 3.70
Ka	14	8	0.89 ± 0.06	3.85 ± 2.10
Ma	21	18	0.99 ± 0.02	5.36 ± 2.80
Sa	17	16	0.99 ± 0.02	4.41 ± 2.30
Ku	13	13	1.00 ± 0.03	5.25 ± 2.80
Ce	19	17	0.99 ± 0.02	4.27 ± 2.20
Bi	20	20	1.00 ± 0.02	5.82 ± 3.00
LS	7	7	1.00 ± 0.08	4.30 ± 2.50
KK	5	5	1.00 ± 0.13	5.07 ± 3.20
Ba	7	7	1.00 ± 0.08	9.01 ± 5.10
Total	247	185		

3. 3. Sequences

Among the 247 individuals of *A. perideraion*, the 400 bp long mtDNA control region sequences revealed 185 different haplotypes from all populations (Table 2). The number of polymorphic sites was 202 and of the number of substitutions 169 were transitions and 49 were transversions. The

distribution of haplotype frequencies reflects the intensity of gene flow through, since shared haplotypes varied among the *A. perideraion* populations (Fig.5). There was no common haplotypes present in most populations, which could indicate low connectivity between the populations. Among all the populations 22 haplotypes resulted to be shared by two or more populations and all others were unique, so over 88% of the individuals had unique haplotypes. Unique refers to those which are only present in one population. The most common haplotypes were haplotype 13, which is carried by 7 individuals and appeared in three populations and the haplotype 33, which is carried by 6 individuals and appeared in five populations (appendix 6).

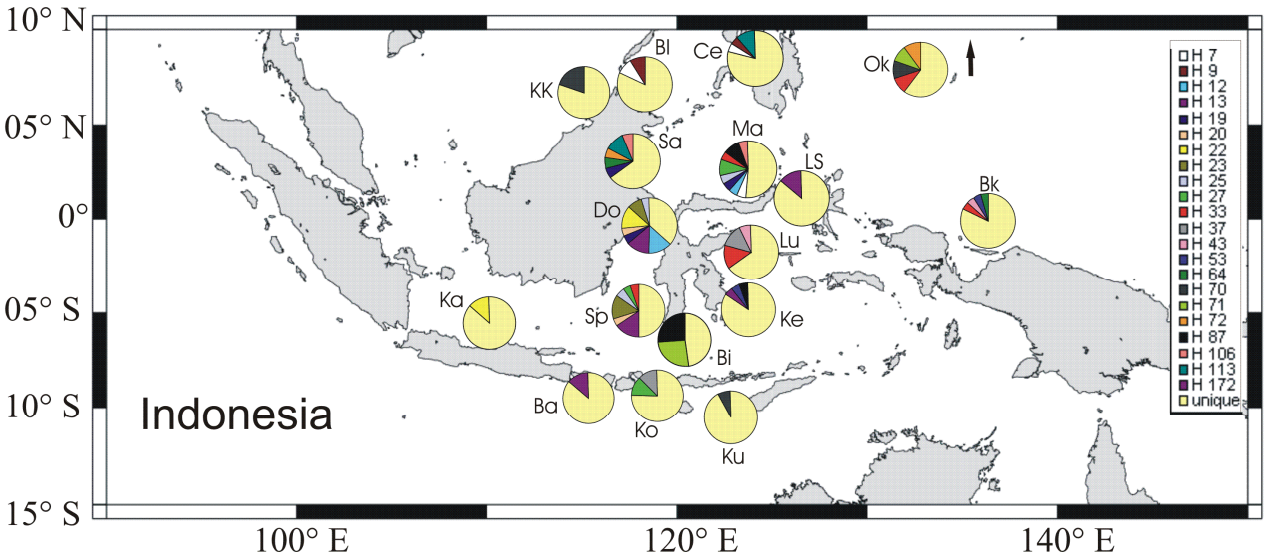


Fig. 5: Haplotypes frequencies of *A. perideraion* in the Indo-Malay Archipelago.The colours in the pie diagrams indicate the proportion of each haplotype (see legend) present in the population.

Abbreviations: Ka – Karimunjava, Ba – Bali, Ko – Komodo, Ku – Kupang, Bi – Bira, Sp – Spermonde Archipelago (Makassar), Do – Donggala, Ma – Manado, LS – Lembeh Strait, Lu – Luwuk, Ke – Kendari, Sa – Sangalaki, KK – Kota Kinabalu (Malaysia), Bl – Banggi Islands (Malaysia), Ce – Cebu (Phillippines), Ta – Taiwan, Ok – Okinawa (Japan), Bk – Biak.

3.4. Selective neutrality tests

Tajima's D is not significant (p>0.05) (Table 3) indicating selective neutrality of the used genetic marker. Therefore it is ideal for the population genetic structure analysis. The significant Fu's F and Fu's and Li's values (p< 0.05) imply either selection or sudden population expansion.

Table 3: Results of selective neutrality tests for *A. perideraion* (all sequences); * = significant; ns =not significant.

Tajima's D	Fu's Fs	Fu's and Li's D
-1.06088 (p = 0.13) ns	-23.60239 (p = 0.01)*	-1.96644 (p = 0.10) ns

3.5. Mismatch distribution

A mismatch distribution analysis under sudden population expansion model gives a unimodal curve (Fig. 6). The test of goodness-of-fit of observed and simulated curves showed no significant difference (p= 0.63) between observed and simulated curves, which indicates a sudden population expansion.

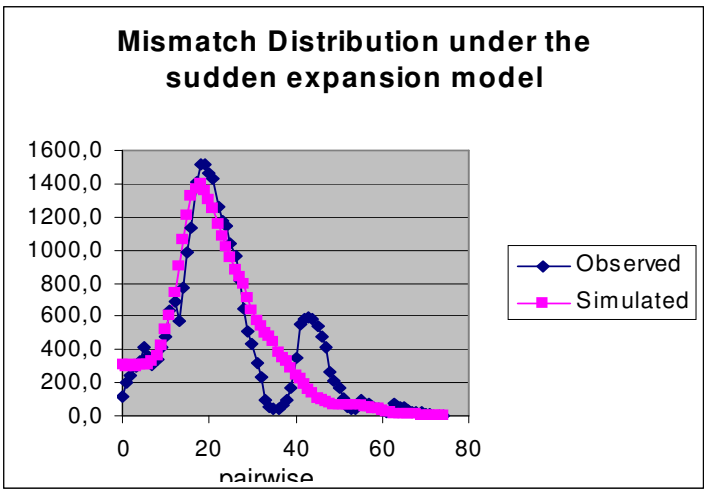


Fig. 6: Mismatch distribution of mtDNA sequences of *A. perideraion* (Y-axis is the frequency of occurrence).

3.6. AMOVA analysis

The total genetic variation was calculated by AMOVA. The Φ_{st} estimate was 0.096 ($p < 0.001$), which indicates restricted gene flow between populations. Therefore, the hypothesis of panmixia was rejected. The studied populations show a genetic structure with higher genetic variation within than among populations (Table 4).

Table 4: AMOVA of *A. perideraion* in the Indo-Malay Archipelago.

Source of variation	d.f	Sum of squares	Variance components	% of variation
Among population	16	440.68	1.15 Va	9.56
Within populations	230	2512.53	10.92 Vb	90.44
Total	246	2953.22	11.16	-------
Φ_{st} = 0.096 **p = 0.001**				

3.7. AMOVA with alternative groupings

Analysis of alternative grouping of *A. perideraion* populations in a hierarchical AMOVA demonstrated that highly significant variation in the data was found by arranging populations into three distinct groups. The highest significant differentiation $\Phi_{ct} = 0.125$ ($p < 0.0003$) was found between the population of karimunjava, the populations of Biak, Kendari, Luwuk and the rest of the populations (Kota Kinabalu, Banggi, Cebu, Okinawa, Lembeh, Manado, Sangalaki, Donggala, Spermando, Bali, Komodo, Kupang, Bira) (Table 5, Fig. 7). This result suggests three distinct populations exist in the Indo-Malay Archipelago.

Table 5: Φ-values of the hierarchical AMOVA of the different populations of *A. perideraion* in the Indo-Malay Archipelago (* = significant p-value).

Groups	Among population within groups (Φ_{sc})	Within population (Φ_{st})	Among groups (Φ_{ct})	Φ_{ct} p-value
(Karimunjava), (Kota Kinabalu, Banggi, Cebu, Okinawa, Lembeh, Manado, Sangalaki, Donggola, Spermando, Bali, Komodo, Kupang, Bira), (Luwuk, Kendari, Biak),	0.038	0.159	**0.125**	**p<0.0003***
(Karimunjava), (Cebu, Banggi, Kota Kinabalu), (Okinawa, Donggala, Spermando, Komodo, Manado, Sangalaki, Kupang, Bira, Lembeh, Bali), (Luwuk, Kendari, Biak),	0.037	0.129	0.096	p<0.001*
(Karimunjava), (Kota Kinabalu), (Banggi, Cebu), (Donggala, Spermando, Komodo, Kupang, Bira, Bali, Lembeh, Manado, Sangalaki, Okinawa), (Luwuk, Kendari, Biak),	0.038	0.130	0.094	p<0.002*
No Grouping			0.096	p<0.001*

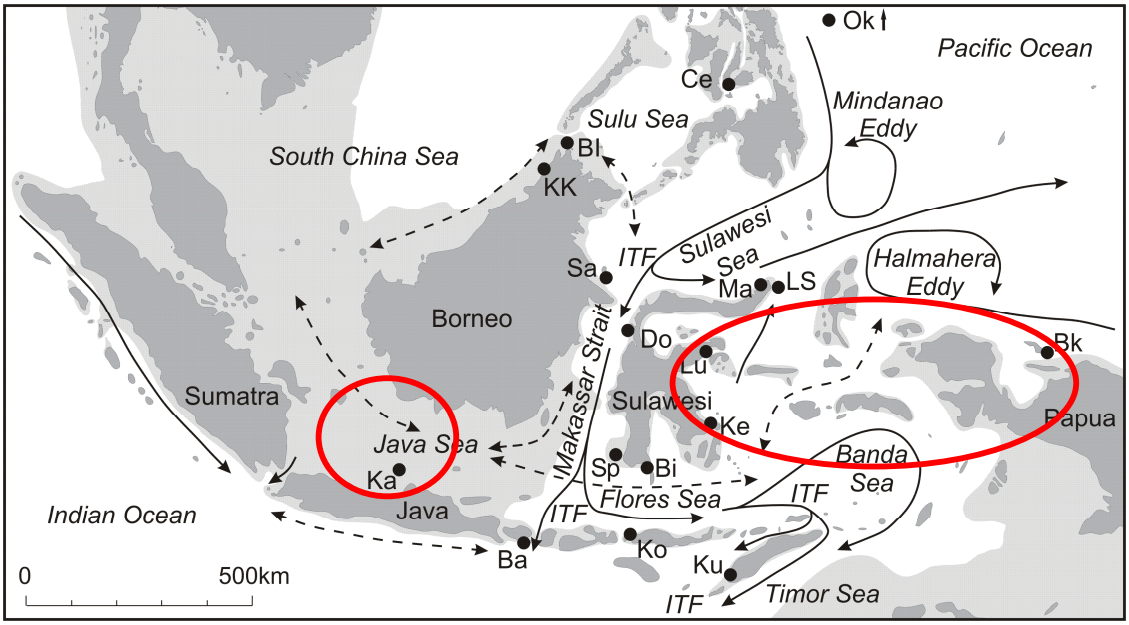


Fig. 7: AMOVA alternative groupings of *A. perideraion* populations. Red circles indicate the population of Ka, the populations of Bk, Ke, and Lu. The rest the populations including Ok is the third group the hierarchical AMOVA. Abbreviations are for populations sites in Fig. (5).

3.8. Pairwise Φ_{st} values between populations

Pairwise Φ_{st} values were significant among almost all populations (Table 6). There were highly significant pairwise Φ_{st} values between the populations from Karimunjava and all other *A. perideraion* populations. There were no significant pairwise Φ_{st} values among the populations from Donggala, Bira, Spermonde and Kota Kinabalu, indicating a low genetic differentiation between these populations. The pairwise Φ_{st} p-values range from one to zero where at one means total isolation and zero indicates no restriction in gene flow. The Significant levels for the pairwise Φ_{st} test was ($p<0.05$)

Table 6: Matrix of pairwise Φ_{st} values and significant p-values between *A. perideraion* populations in the Indo-Malay Archipelago. (ns = not significant; significant levels: ***=p<0.0001, **= p<0.001, *=p<0.01).

	BI	Do	Sp	Ko	Lu	Bk	Ok	Ke	Ka	Ma	Sa	Ku	Ce	Bi	LS	KK	Ba
BI		**	**	*	**	***	**	***	***	ns	ns	*	*	ns	ns	ns	**
Do	0.13		ns	*	**	***	***	**	***	**	**	**	**	*	**	ns	**
Sp	0.14	0.02		ns	**	***	Ns	*	***	*	*	*	ns	ns	ns	ns	*
Ko	0.06	0.06	0.02		ns	**	Ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns
Lu	0.11	0.13	0.12	0.05		ns	*	ns	**	**	*	ns	**	ns	ns	ns	ns
Bk	0.21	0.21	0.20	0.16	0.00		***	ns	***	***	***	**	***	**	***	ns	ns
Ok	0.13	0.15	0.04	0.01	0.09	0.18		**	***	**	ns	ns	ns	*	ns	ns	ns
Ke	0.15	0.13	0.09	0.06	0.01	0.00	0.09		***	**	**	*	**	*	ns	ns	ns
Ka	**0.21**	**0.21**	**0.20**	**0.15**	**0.18**	**0.26**	**0.21**	**0.20**		***	***	***	***	***	***	***	***
Ma	0.06	0.07	0.05	0.02	0.12	0.21	0.09	0.11	**0.15**		ns	*	ns	*	ns	ns	*
Sa	0.01	0.08	0.07	0.03	0.08	0.19	0.04	0.11	**0.16**	0.03		*	ns	ns	ns	*	*
Ku	0.06	0.12	0.08	0.02	0.04	0.14	0.05	0.08	**0.12**	0.06	0.03		*	ns	ns	ns	ns
Ce	0.05	0.13	0.05	0.02	0.10	0.20	0.00	0.10	**0.19**	0.04	0.01	0.05		ns	ns	ns	ns
Bi	0.04	0.03	0.04	0.01	0.04	0.12	0.06	0.05	**0.15**	0.04	0.00	0.03	0.03		ns	ns	ns
LS	0.05	0.14	0.08	0.01	0.08	0.17	0.02	0.09	**0.19**	0.07	0.01	0.01	0.01	0.02		ns	ns
KK	0.10	0.13	0.02	0.05	0.10	0.17	0.02	0.08	**0.20**	0.05	0.05	0.04	0.01	0.07	0.03		ns
Ba	0.11	0.13	0.08	0.01	0.01	0.03	0.03	0.01	**0.17**	0.09	0.07	0.02	0.06	0.03	-0.04	0.01	

3.9. Haplotype-network

In the haplotype network (Fig. 8; Appendix 7) the number of substitutions (mutations) separating haplotypes was remarkably high and haplotypes grouped into 6 fairly distinct clades, with the rest of the haplotypes attached singly to one of these 6 clades with varying stepwise mutational distances. Each colour in the network corresponds to a clade. There is no obvious geographic pattern shown in the clustering. However, there are also very big mutational steps between some of the clades. Most haplotypes were separated by 6 or 10 mutational steps, but there are also distinct groups that have fewer mutational steps between haplotypes within the group than between this and the other groups. The groups were separated by 13 or more mutational steps, but two haplotypes were separated by 53 and 58 steps from its nearest relative.The frequencies of the clades represented in each population of *A. perideraion* in the Indo-Malay Archipelago (Fig. 10) showed that Karimumjava and Banggi Islands population haplotypes are represented only in the blue clade (A), and the light pink clade (F) was, with one single sequence, exclusive to Bali population in Southern Indonesia. Okinawa, Japan and the Phillippines have a proportion of the dark violet and blue clades in their populations. The Biak, Luwuk and Kendari populations had light blue clade haplotypes (D) in common. These results indicate the isolation of Karimunjava from the rest of the populations studied here and support the hierarchical AMOVA grouping.

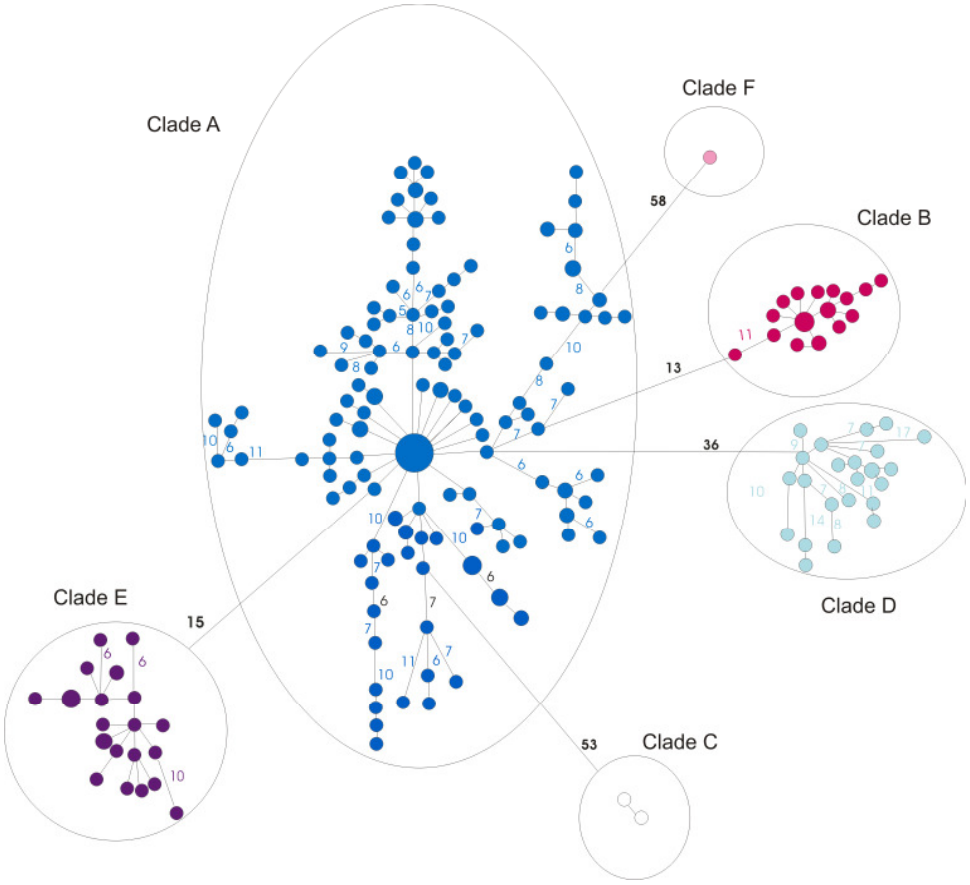


Fig. 8: Haplotype network for *A. perideraion* in the Indo-Malay Archipelago. Small circles indicate haplotypes, the small size circle indicates one individual and big circle indicates more than one individual carried the specific haplotypes. Haplotypes belonging to specific clades are encircled. Lines between haplotypes indicate one mutation between tow haplotypes and numbers on lines indicates additional mutation between observed haplotypes.

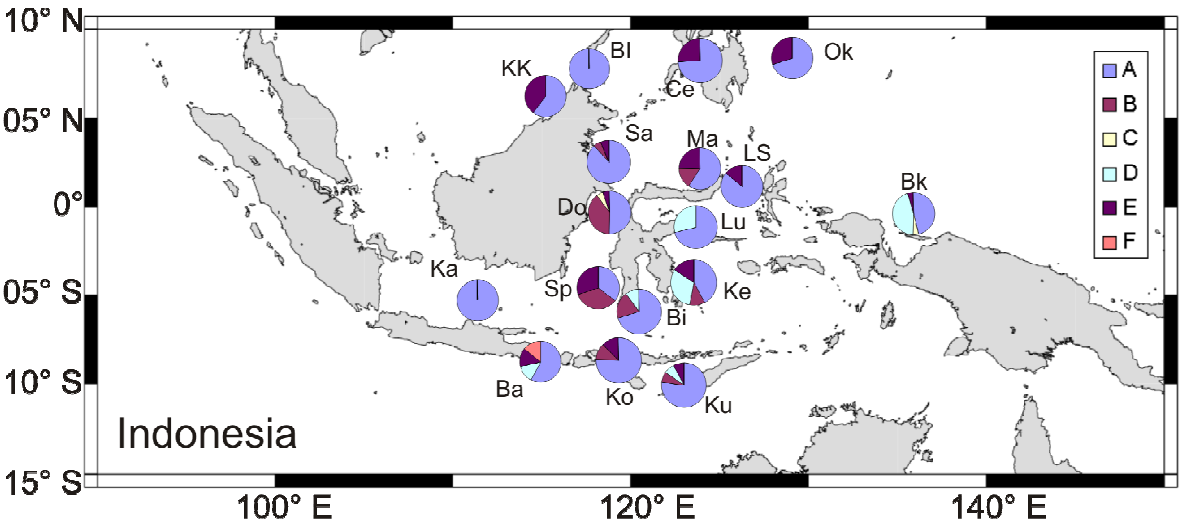


Fig. 9: Pie diagrams showing frequencies of clades of mtDNA control region sequences represented in each population of *A. perideraion* in the Indo-Malay Archipelago including Okinawa, Japan (names of clades see legend; abbreviations for populations are in Fig. 5).

3.10. Genetic and geographic distances (IBD)

The correlation between populations pairwise Φ_{st} and geographical distance in all population was not significant($r = 0.1879$, $p < 0.1370$). The results imply that there is no correlation between the genetic differences and the geographic distance.

4. Discussion

The result of the present study detected a strong genetic population structure, mainly caused by restricted gene flow between populations of *A. perideraion* in the Indo-Malay Archipelago. This was expected due to the life history of *A.perideraion,* having a short planktonic larval durations and sedentary adults. Partly, the genetic population structure could be explained by the separation of ocean basins during sea level low stands of the Pleistocene (Nelson et al 2000). Such restricted gene flow across the Indo-Malay Archipelago has only been reported in few other coral reef species: the shallow water sea urchin, *Tripneustes* (Lessios *et al.* 2003; Lessios *et al.* 2001), the widely distributed goatfish, *Mulloidichthys vanicolensis,* (Stepien *et al.* 1994) and many coral reef fish species with dispersing larvae (Bernardi *et al.* 2001; Planes and Fauvelot 2002; Rocha *et al.* 2002; Taylor and Hellberg 2003, 2005; Bay *et al.* 2004; Messmer *et al.* 2005; Nelson *et al.* 2000; Barber *et al.* 2002a, 2002b; Borsa 2003). Tajima's test was not significant, which indicates neutrality of the used genetic marker, the mtDNA control region. Therefore, it is ideal for population genetic structure analysis (Palumbi and Baker, 1994; FitzSimmons et al, 1997). The significant Fu's F and Fu and Li's F and D values imply either selection or sudden population expansion of the populations of *A. perideraion* (Slatkin and Hudson 1991). Since mtDNA is uniparentally inherited, its effective population size is four-fold smaller than that of nuclear genes (Palumbi and Baker 1994; FitzSimmons et al.1997). MtDNA is therefore more sensitive to demographic events such as reduction in population size and geographic isolation. Therefore, a

Mismatch Distribution Analysis was applied and the plotted mismatch distribution for the entire sample of *A. periderion* was done to determine if the observed distribution of the average number of pairwise sequence differences corresponded with the expected distribution. A non-significant result suggests population expansion. According to Excoffier (2004) it is feasible and it is quite likely that many natural populations have experienced expansion due to sea level rise after a separation or reduction of suitable habitat. The population expansions may result in mixing of previously isolated populations (Ray *et al.* 2003; Excoffier 2004), as was noted in the widely distributed parrotfish, *Chlorurus sordidus* (Bay *et al.* 2004). Bottlenecks followed by population expansions may leave a genetic signature in extant populations (Grant and Bowen 1998).

Significant genetic differences in pairwise comparisons were found between *A. perideraion* populations from the Sunda Shelf (Karimunjava) and those from the rest of the Indo-Malay Archipelago, including South China Sea, Bali Strait, Sulu Sea and Sulawesi Sea. Gene flow in *A. perideraion* was inferred to be restricted between most populations. However, gene flow in the Pink Anemonefish was assumed to be higher between regions connected by the permanent current that flows through the Makassar Strait from the Sulawesi Sea to the Bali Strait (Indonesian Throughflow: Fieux *et al.* 1994). Previously, it was reported that populations of snapper (*Pristipomoides multidens*) showed geographic structure between northern Australia, Indonesia and West Papua (Ovenden *et al.* 2004). This was attributed to constraints to dispersal at all life stages. Constraints to dispersal that contribute to, and maintain, the observed degree of genetic differentiation are experienced presumably by all life history stages of this tropical marine fish. The observed population structure of *A. perideraion* is consistence with those studies. Alternative grouping of populations in the Indo-Malay Archipelago showed at least three groups of populations identified as the following; Karimunjava (Sunda Shelf/Java Sea); Luwuk, Biak and Kendari (East Sulawesi and New Guinea); and the rest of the Indo-Malay Archipelago. Sound fishery management commands that these three geographically delineated, genetically differentiated populations be considered as separate management units.

Although some species exhibit genetic uniformity over broad geographic ranges as predicted, other species show strong genetic differentiation among populations from different geographic regions, indicating that larval dispersal can

be more limited than is believed. Moreover, a recent focus on dispersal as a significant process in ecology and evolutionary biology specifically in the marine environment (Palumbi 2003, 2004) has provided an additional impetus for the genetic analysis of population structure in widely distributed marine species. Much of the previous work on population genetics and larval dispersal of marine fishes has primarily been concerned with species distributed in Pacific, Indian, and Indo-West Pacific Oceans. Few to rare studies have been reported in the central Indo-Malay Archipelago. Therefore, there are recommendations for further studies on the factors that impact the ecology and life-histories of adults and larvae of a wide variety of marine vertebrate and invertebrate species to reveal patterns of barriers and connectivity in the Indo-Malay Archipelago.

Studies in Indonesian waters, a region with a pronounced physical oceanography (Fig.10), have shown that the strong currents of the Indonesia result in connectivity between populations of the marine species (Barber *et al.* 2000). A restriction in gene flow could be detected between population that are not connected by major currents and predictions of limited connectivity across the Molucca Sea based on limited water transport resulting from the Halmahera Eddy (Fig. 10). The result of the present study showed that East Sulawesi is closer connected to New Guinea, than to close by populations of West Sulawesi is in agreement with those studies.

Surface currents in the western part of the Indo-Malay Archipelago seasonally vary according to typical monsoon cycles (Fig.10; Wyrtki, 1961). The monsoon cycle induces shifts in water circulation, which coincide with the seasonal migration of adults and influence the dispersion of the larvae of pelagic fishes (Hardenberg 1937). However, the high connectivity of the seas in the Indo-Malay Archipelago may favor high levels of gene flow between populations which was not the case with the species studied here.

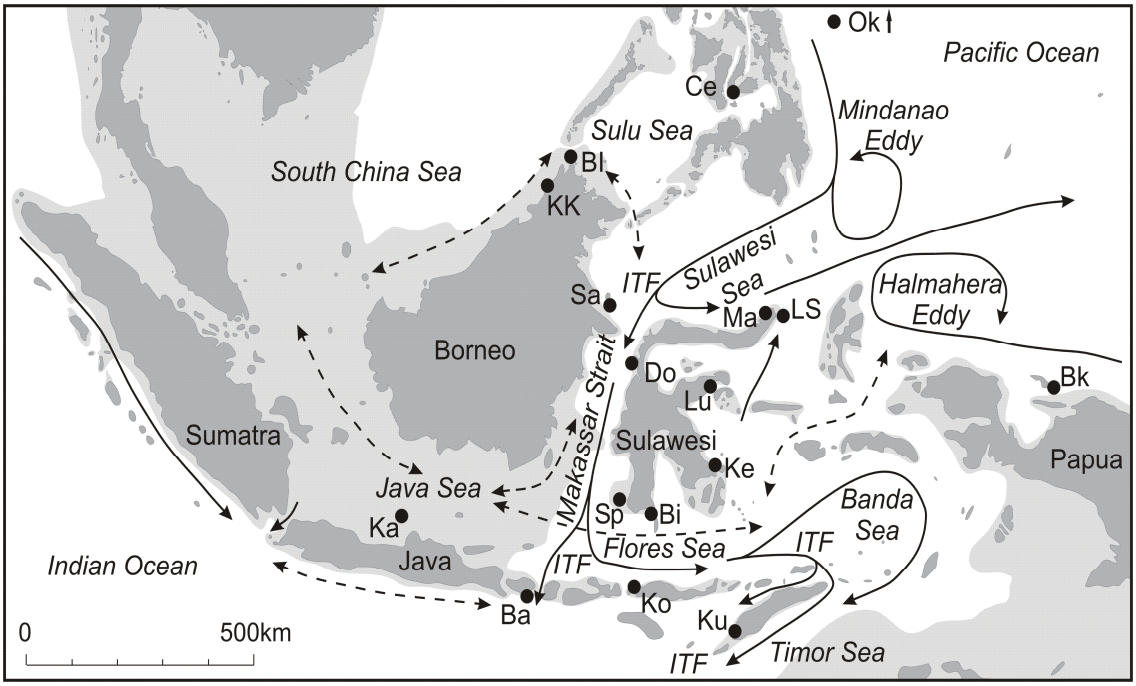


Fig. 10: Indo-Malay archipelago map showing dominant surface currents (bold arrows); seasonal surface currents (dotted arrows) (after Wyrtki, 1961) and sampling locations (dark circles). Land areas (shaded) at the apogee of Pleistocene glaciations were inferred from present bathymetric charts and from using coastline of 120 m below present sea level (Voris, 2000). Abbreviations for populations are in Fig .5.

At the highest point of Pleistocene glaciations, when most continental seas were above sea level, the habitat of coastal fishes was considerably reduced, implying much smaller population sizes than at present. The rise in sea level and the concomitant expansion of potentially favorable habitat may have allowed population expansion and the reclaiming of shallow seas such as the Java Sea. This study suggests that *A. perideraion* populations retain evidence of isolation between locations since the Miocene, when sea levels were intermittently reduced. This was followed by expansion when sea levels were intermittently elevated again and at the end of the Pleistocene, sea levels repeatedly dropped substantially below present day levels (Hallam 1984; Miller *et al.* 2005) resulting in isolation among previously continuous habitats. The result of Isolation by Distance Analysis assesses whether the pairwise genetic distance is correlated with the geographic distance (Rousset 1997, Hutchinson and Templeton 1999). In this study the data showed no evidence of isolation by distance in agreement

with some other widespread reef species (Klanten *et al*. 2007). This suggests that other factors are involved in the genetic differentiations in *A. perideraion*. According to Chenoweth *et al.* (1998) the sea level changes have left a significant imprint on the molecular diversity and the population genetic structure within marine species in the Indo-West Pacific. Among the mtDNA haplotypes of *A. periderion*, the haplotype network showed six distinct clades. The highest divergence was between 13 and 58 mutational steps, found in Bali and Biak. The eastern populations (those represented by samples from Biak, Luwuk and Kendari) have, next to clade (A) and (C), a large proportion of clade D haplotypes. Only Bali, Bira and Komodo show also small proportions of the latter clade. The westernmost population Karimunjava possesses clade (A) only. Only the intermediate populations sampled in Makassar Strait and the one found in northern and southern parts have clades (E) and (F) together with clade (A). These observations indicate past geographic isolation of the most eastern and most western populations, that could have been followed by reproductive isolation as reported in other marine fishes (e.g., Hoarau and Borsa, 2000; Borsa and Quignard 2001; Pfeiler *et al*. 2002). However, the presence of geographic association of mtDNA control region clades to particular ocean basins may be explained by contrasts strongly with others as was found by Ely *et al.* (2005) for some pelagic species (skipjack tuna and yellowfin tuna). In this study each clade consisted of individuals sampled from throughout the Indo-Malay distribution range of *A. perideraion*, mixed clades, indicating limited gene flow to some regions, which is partly through history and partly through current patterns. This result suggests that there was no clear break for genetic difference in the relative grouping in the northern and southern populations of this species and this was not the case for the other species studied in this area. For example, phylogeographic studies focussed on particular groups, primarily pomacentrids (Doherty *et al.* 1994; Planes *et al.* 2001) and gobiids (Taylor and Hellberg 2003) have demonstrated localized population structures in these reef fishes. Through the Pleistocene, the Sunda and Sahul Shelves were repeatedly exposed above sea level, constituting land barriers between the northeastern Indian Ocean and the western Pacific Ocean. The Java Sea was completely above sea level, the South China Sea was completely isolated from the Indian Ocean in its South and from the Sulu Sea in its East, and the Makassar Strait was much narrower than the present also the Banda Sea (Voris 2000), presumably restricting the oceanic

flow between the Sulawesi Sea and the rest of the Indo-Malay Archipelago. The pattern of Sulawesi Sea populations being isolated by Pleistocene sea levels has been suggested for a mantis shrimp (Barber *et al.* 2000, 2002) and for the False Clown Anemonefish on either side of the Malay Peninsula (Nelson *et al.* 2000) as well as between barramundi populations on either side of Torres Strait (Chenoweth *et al.*1998). This apparently recurrent feature of the mitochondrial phylogenies and population genetic analyses of coastal fishes and invertebrates in the Indo-Malay region lends support to the hypothesis that changes in sea level associated with Pleistocene glaciations provoked the geographic isolation of populations in the Indo-Malay region.

Genetic divergence between geographically isolated populations might have reached a level associated with possible reproductive isolation in coastal, sedentary species such as butterflyfishes (Mc Millan and Palumbi 1995). However, other fish species, like the Pink Anemonefish, might have experienced shorter durations of geographic isolation. This isolation could have prevented total reproductive isolation; hence secondary contact would have been possible when the sea level rose again. The Karimunjava population was genetically distinct from all other populations in this study. The lower nucleotide diversity for the used mitochondrial marker, the presence of a unique haplotype as well as the less frequency of the shared haplotypes are consistent with the hypothesis that a substantial population bottleneck occurred during or after the founding of this population. Either few individuals established the population or the effective population size was reduced. In order to decide that further research and investigations is needed.

5. Conclusions

The aim of the thesis was to investigate the population structure of the ornamental fish species *A.perideraion* in the Indo-Malay Archipelago by analyzing the sequences of the molecular marker mtDNA control region (400 bp) and to suggest if it is a neutral gene for population structure analysis. Significant population structure (Φ_{st} = 0.096) was found, indicating there is restricted gene flow between the populations. The pairwise comparison of the populations shows a high differentiation. The strong genetic differentiation of the Karimunjava population compared to the rest of the *A. perideraion* populations could be due to

the history of the Sunda self during low sea level stands. The Low genetic differentiation between the population of Donggala, Bira and Spermonde compared to the other populations could be due to the Indonesian through-flow current effects on larval dispersal by stepping stones. Furthermore, a significant divergence between the populations of East Sulawesi and New Guinea (Biak, Luwuk, Kenderi), the Java Sea as the western most population (Karimunjava), and middle parts of the Indo-Malay Archipelago was found. However, there is no correlation between the genetic and geographic distances through Isolation by Distance. Thus, given the relatively short pelagic larval duration of this species, coupled with the regular occurrence of the Indonesian Throughflow (ITF) currents associated with strong currents, alternating with the monsoon seasons, the population structure of *A. perideraion* in the Indo-Malay archipelago is found in the present study.

This work represents a first look at the population genetics of one ecologically and economically important ornamental fish species *A. perideraion* in the Indo-Malay Archipelago. In spite of the fact that the structure of other Indo-Malay coral reef fish species remains unexplored, this work is a first step towards a better understanding of the movement of larvae in the Indo-Malay Archipelago for this fish. A better insight of the significance of larval dispersal and connectivity between populations to localized differentiation is critical at this point because the ornamental fish is heavily exploited in this region. Furthermore, understanding larval dispersal and the resulting population genetic structure of *A. perideriaon* is critical to fisheries management through marine reserves in order to ensure connectivity and sustainability of the *A. perideraion* and other anemonefish populations for the future. Results from this study suggest that this fish species should be considered as three different stocks for management.

6. References

Allen G., (1975). The anemone fishes. Their classification and biology. Second edition. Neptune City, New Jersey: T.F.H. Publications.

Allen G., (1991). Damselfishes of the world. Melle, Germany: Mergus Publishers.

Arvedlund, M., M. McCormick, and T. Ainsworth. (2000). Effects of Photoperiod on Growth of Larvae and Juveniles of the Anemonefish *Amphiprion melanopus*. Naga: The ICLARM Quarterly, Vol. 23 (2): 18-23.

Avise J. C., Arnold J., Ball R. M.,Bermingham E., Lamb T., Neigel J. E., Reeb C. A., and Saunders N. C. (1987). Intraspecific phylography: the mitochondrial DNA bridge between population genetics and systematics. Annu. Rev. Ecol. Syst., Vol. (18): 489-522.

Akihisa H., (2000). Social and mating systems of the protandrous anemonefish *Amphiprion perideraion* under the influence of a larger congener. Austral Ecol., Vol. 25 (2): 187–192.

Armsworth P. R., James,M.K., and Bode, L., (2001).When to press on or turn back: dispersal strategies for reef fish larvae. Am. Nat., Vol. 157: 434-450.

Balon E., (1990). Epigenesis of an epigeneticist: the development of alternative concepts on the early ontogeny and evolution of fishes. Guelph Ichthyol. Rev., Vol.1: 1-48.

Barber P. H., Palumbi S. R., Erdmann M. V. and Moosa M. K., (2002a). Sharp genetic breaks among populations of *Haptosquilla pulchella* (Stomatopoda) indicate limits to larval transport: patterns, causes, and consequences. Mol. Ecol., Vol. 11: 659−674.

Barber P. H., Moosa M. K. and Palumbi S. R. (2002b). Rapid recovery of genetic diversity of stomatopod populations on Krakatau: temporal and spatial scales of marine larval dispersal. Mol. Ecol., Vol. 11: 659–674.

Barber, P. H., S. R. Palumbi, M. V. Erdmann, and M. K., Moosa (2000). Biogeography. A marine Wallace's line? Nature. Vol., 406:692–693.

Bay L. K., Choat J. H., van Herwerden L, Robertson D. R., (2004). High genetic diversities and complex genetic structure in an Indo-Pacifc tropical reef fish (*Chlorurus sordidus*): evidence of an unstable evolutionary past? Mar. Biol., Vol. 144:757–767.

Benzie J. A. H., Ballment E., Forbes A. T., Demetriades N. T., Sugama K., Haryanti and Moria S., (2002). Mitochondrial DNA variation in Indo-Pacific populations of the giant tiger prawn, *Penaeus monodon*. Mol. Ecol., Vol. 11: 2553−2569.

Berardi G., Holbrook S. J., and Schmitt S. J., (2001).Gene flow at three apatial scales in a coral reef fish, the three-spot dascyllus, *Dasyllus trimaculatus*. Mar. Biol., Vol. 138: 457-465.

Birky C. W. J., Fuerst P. and Maruyama T., (1989). Organelle gene diversity under migration, mutation, and drift: equilibrium expectations, approach to

equilibrium, effects of heteroplasmic cells, and comparison to nuclear genes. Genetics, Vol.121:613-627.

Black K. P., (1993). The relative importance of local retention and inter-reef dispersal of neutrally buoyant material on coral reefs. Coral Reefs, Vol.12: 43-53.

Bohonak A. J., (1999). Dispersal, gene flow, and population structure. Quart. Rev. Biol, Vol. 74 (1): 21–45.

Borsa P., (2003) Genetic structure of round scad mackerel *Decapterus macrosoma* (Carangidae) in the Indo-Malay archipelago. Mar. Biol., Vol.142: 575−581.

Borsa P., and Quignard J. P., (2001). Systematics of the Atlantic-Mediterranean soles *Pegusa impar*, *P. lascaris*, *Solea aegyptiaca*, *S. senegalensis*, and *S. solea* (Pleuronectiformes: Soleidae). Can. J. Zool., Vol. 79: 2297−2302.

Boyer, M., P. Bearzi, F. Ricciardi. 2004. "Pink anemone fish" (On-line). Accessed October 18, 2005 at: http://www.edge-of-reef.com/pomacentridi/ clownfishen.htm

Boyer, S. 2005. "Florida Museum of Natural History Ichthyology Department" (On-line). Accessed October 18, 2005 at http://www.flmnh.ufl.edu/fish/ Gallery/Descript/PinkAnemonefish/PinkAnemonefish.

Brown W. M., (1985). The mitochondrial genome of animals. Pages 95-130 in R.J. MacIntyre, editor. Molecular evolutionary genetics. New York, Plenum Press.

Benzie J. A. H., Smith C., and Sugama K., (2003). Mitochondrial DNA reveals genetic differentiation between australian and indonesian pearl oyster *Pinctada maxima* (jameson 1901) populations. J. Shellfish Res., Vol. 22, No. 3, 781–787.

Clayton D. A., (1982). Replication of animal mitochondrial DNA. Cell 28:693-705.

Chang D. D., and Clayton D. A., (1986). Identification of primary transcription start sites of mouse mitochondrial DNA: accurate in vitro initiation of both heavy- and light-strand transcriptions. Mol. Cell. Biol., Vol. 6:1446-1453.

Cesar H., (1996). Economic Analysis of Indonesian Coral Reefs. Environment Dept, World Bank. 97pp.

Chenoweth S. F., Hughes J. M., Keenan C. P., Lavery S., (1998). When oceans meet: a teleost shows secondary intergradation at an Indian-Pacific interface. Proc. R. Soc. Lond., B 265: 415−420.

Coughlin D. J., Strickler J. R. and Sanderson B., (1992). Swimming and search behaviour in clownfish, *Amphiprion perideraion*, larvae. J. Anim. Behav., Vol. 44: 427-440.

Cowen R. K., (1985). Large scale pattern of recruitment by the labrid, Semicossyphus pulcher: Causes and implications. J. Mar. Res., Vol., 43: 719-742.

Cowen R. K., Lwiza K. M. M., Sponaugle S., Paris C. B., Olson D. B., (2000). Connectivity of marine populations: open or closed? Science 287:857-859.

Cowen R. K., (2002). Larval dispersal and retention and consequences for population connectivity. Pages 149-170 in P. F. Sale, editor. Coral Reef Fishes. New York, Academic Press.

Darling K. F., Wade C. M., Stewart I. A., Kroon D., Dingle R., Brown A. J. L., (2000). Molecular evidence for genetic mixing of Arctic and Antarctic subpolar populations of planktonic foraminifers. Nature, Vol., 405:43.

Doherty P. J., Mather P., Planes S., (1994). Acanthochromis polyacanthus, a fish lacking larval dispersal, has genetically differentiated populations at local and regional scales on the Great Barrier Reef. Mar. Biol., Vol.12:11-21.

Duda T. F., and Palumbi S. R., (1999). Population structure of the black tiger prawn, Penaeus monodon, among western Indian Ocean and western Pacific populations. Mar. Biol., Vol. 134 (4): 705-710.

Ekman, S., (1953). Zoogeography of the sea. Sidwick and Jackson, London.

Ellegren H., (2000). Microsatellite mutations in the germline: implications for evolutionary inference. Trends Genet., Vol. 16: 552-559.

Ely B., Viñas J., Alvarado Bremer J.R., Black D., Lucas L., Covello K., Labrie A. V., Thelen E., (2005). Consequences of the historical demography on the global population structure of two highly migratory cosmopolitan marine fishes: the yellowfin tuna (*Thunnus albacares*) and the skipjack (*Katsuwonus pelamis*). BMC, Evol. Biol., Vol., 5:9.

Excoffier L., (2004). Patterns of DNA sequence diversity and genetic structure after a range expansion: lessons from the infinite-island model. Mol. Ecol., 13:853–864.

Fautin D. G.. (1992). Anemonefish recruitment: the roles of order and chance. Symbiosis, Vol. 14: 143-160.

Fautin D. G. and Allen G. R., (1992). Anemone fishes and their host sea anemones, a guide for aquarists and divers. Western Australian Museum, Perth. 160pages.

Fieux M., Andrié C., Delécluse P., Ilahude A. G., Kartavtseff A., Mantisi F. (1994). Measurements within the Pacific−Indian oceans throughflow region. Deep-Sea Res., Vol. 41: 1091−1130. FitzSimmons N. N., Moritz C., Limpus C. J., Pope L., Prince R., (1997). Geographic structure of mitochondrial and nuclear gene polymorphisms in Australian green turtle populations and male-biased gene flow. Genetics, Vol. 147: 1843−1854.

Fisher R., Bellwood D. R., and Job S. D., (2000). Development of swimming abilities in reef fish larvae. Mar. Ecol. Prog. Series, 202:163-173.

Fu Y. X., (1997). Statistical tests of neutrality of mutations against population growth, hitch-hiking and background selection. Genetics, Vol.147, 915–925.

Godfrey J. S., (1996). The effect of the Indonesian throughflow on ocean circulation and heat exchange with the atmosphere: A review, J. Geophys. Res., Vol. 101: 12217–12238.

Gordon A. L., and Fine R. A., (1996). Pathways of water between the Pacifi c and Indian Oceans in the Indonesian seas. Nature, Vol. 379:146–149.

Grant W. S., and Bowen B. W., (1998). Shallow population histories in deep evolutionary lineages of marine fishes: insights from sardines and anchovies and lessons for conservation. J. Hered, Vol. 89: 415−426.

Gopurenko D. and Hughes J. M., (2002). Regional patterns of genetic structure among Australian populations of the mud crab, Scylla serrata (Crustacea: Decapoda): evidence from mitochondrial DNA. Mar. Freshw. Res., Vol. 53: 849−857.

Hallam A., (1984). Pre-Quaternary sea-level changes. Annu. Rev. Earth. Planet Sci., Vol. 12: 205–243.

Hall T. A., (1999). Bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series, 41: 95–98.

Hewitt G. (2000). The genetic legacy of the Quaternary ice ages. Nature, Vol. 405: 907−913.

Hardenberg J. D. F., (1937). Preliminary report on a migration of fish in the Java sea. Treubia, Vol.16: 295−300.

Hoarau G & Borsa P., (2000). Extensive gene flow within sibling species in the deep-sea fish Beryx splendens. C R Acad. Sci. Sér III 323: 315−325.

Hutchinson D. W., and Templeton A. R., (1999). Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution, Vol. 53: 1898-1914.

Jones G.P., Milicich M. J., Emslie J. J., Lunow C., (1999). Self-recruitment in a coral reef fish population. Nature, Vol. 402:802–804.

Klanten O., Choat J., Herwerden, Lynne, (2007). Extreme genetic diversity and temporal rather than spatial partitioning in a widely distributed coral reef fish. Mar. Biol., Vol. 150 (4), pp.:659-670.

Kuiter, R., Tonozuka. (2001). Pictorial guide to Indonesian reef fishes. Part 2. Fusiliers - Dragonets, Caesionidae - Callionymidae. Australia: Zoonetics.

Kimura M., (1968). Evolutionary rate at the molecular level. Nature 217:624-626.

Lourie S. A., Green D. M., Vincent A. C. J., (2005). Dispersal, habitat differences, and comparative phylogeography of Southeast Asian seahorses (Syngnathidae: *Hippocampus*). Mol. Ecol., Vol. 14 (4): 1073.

Lieske E., and Myers R., (1994). Reef fishes of the world. Periplus Editions, Hong Kong.

Lee W. J.,Conry J.C., Howell W. H., Kocher T. D., (1995). Structure and evolution of Toleost mitochondrial control region. J. Mol. Evol., Vol. 41: 54-66.

Leis J. M., Sweatman H. P.A., Reader S. E., (1996). What the pelagic stages of coral reef fishes are doing out in blue water: daytime field observations of larval behavioural capabilities. Mar. Freshw. Res., Vol. 47:401-411.

Leis, J.M., and M.I. McCormick (2002). The biology, behavior, and ecology of the pelagic larval stage of coral reef fishes. Pp. 171-199 in P.F. Sale, editor. Coral Reef Fishes. New York, Academic Press.

Lessios H. A., Kessing B. D., and Pearse J. S., (2001). Population structure and speciation in tropical seas: global phylogeography of the sea urchin *diadema.* Evolution, Vol. 55: 955–975.

Lessios H. A., Kane J., and Robertson D. R., (2003). Phylogeography of the pantropical sea urchin *Tripneustes*: contrasting patterns of population structure between oceans. Evolution, Vol. *57:* 2026–2036.

Lee T., Fukumori I., Menemenlis D., Xing Z., and Fu L., (2002). Effects of the Indonesian throughfl ow on the Pacific and Indian Oceans. Journal of Physical Oceanography, Vol. 32, pp: 1404–1429.

Mc Millan W. O. and Palumbi S. R., (1995). Concordant evolutionary patterns among Indo-West Pacific butterflyfishes. Proceeding of the Royal of London series B. Biol. Sci., Vol. 260: 229−236.

Messmer V., van Herwerden L., Munday P., Jones G., (2005) Phylogeography of colour polymorphism in the coral reef fish *Pseudochromis fuscus*, from Papua New Guinea and the Great Barrier Reef. Coral Reefs, 24:392–402.

Meyer A., (1991). Evolution of mitochondrial DNA in fish, in Biochemistry and Molecular Biology of Fish, Vol. 2: 1-38.

Miller K. G., Kominz M. A., Browning J.V., Wright J. D., Mountain G. S., Katz M. E., Sugarman P.J., Cramer B.S., Christie-Blick N., Pekar S. F., (2005). The phanerozoic record of global sea-level change. Science, 310:1293–1298.

Mora C, Chittaro P.M., Sale P. F., Kritzer J. P., Ludsin S. A. (2003). Patterns and processes in reef fish diversity. Nature, Vol. 421: 933−936.

Nelson J. S., Hoddell R. J., Chou L. M., Chan W. K., Phang V. P. E., (2000). Phylogeographic structure of false clownfish, Amphiprion ocellaris, explained by sea level changes on the Sunda shelf. Mar. Biol., Vol.137: 727−736.

Ngan E. C., (2006). Isolation by distance web service with incorporation of DNA set. (http://ibdws.sdsu.edu/IBDWS_v23_paper.pdf)

Ovenden J. R, Salini J., O'Connor S. and Street R., (2004). Pronounced genetic population structure in a potentially vagile fish species (Pristipomoides multidens, Teleostei, Perciformes, Lutjanidae) from the East Indies triangle. Mol. Ecol., Vol. 13: 1991−1999.

Pfeiler E., Colborn J, Douglas MR & Douglas M. E., (2002). Systematic status of bonefishes (*Albula* spp. from the eastern Pacific Ocean inferred from analyses of allozymes and mitochondrial DNA. Env. Biol. Fishes, Vol. 63: 151−159.

Planes S., Doherty P. J., and Bernardi G., (2001). Strong genetic divergence among populations of a marine Wsh with limited dispersal, Acanthochromis polyacanthus, within the Great Barrier Reef and the Coral Sea. Evolution, Vol. 55:2263–2273.

Planes S., (2002). Biogeography and larval dispersal inferred from population genetic analysis. Pages 201-220 in P.F. Sale, editor. Coral Reef Fishes. New York, Academic Press.

Planes S., Fauvelot C., (2002). Isolation by distance and vicariance drive genetic structure of a coral reef Wsh in the PaciWc Ocean. Evolution, 56:378–399.

Planes S., Parroni M., Chauvet C., (1998). Evidence of limited gene flow in three species of coral reef fishes in the lagoon of New Caledonia. Mar. Biol., Vol.103: 361-168.

Palumbi S. R., (2004). Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annu. Revi. Environ. Resour., Vol. 29:31–68.

Palumbi S. R., (2003a). Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl., Vol. 13:146–158.

Palumbi S. R., (2003b). Population genetics, demographic connectivity, and the design of marine reserves. Ecol. Appl., Vol. 13(1): 146-158.

Palumbi S. R., and Warner R. R., (2003). Why gobies are like hobbits. Science, Vol. 299: 51-52.

Palumbi S.R. and Baker C. S., (1994). Contrasting population structure from nuclear intron sequences and mtDNA of humpback whales. Mol. Biol. Evol., Vol. 11: 426−435.

Palumbi S. R., (1994). Genetic divergence, reproductive isolation, and marine speciation. Annu. Rev. Ecol. Syst., Vol. 25:547–572.

Parker P.G., Snow A. A., Schug M. D., Booton G. C.,Fuerst P. A., (1998). What molecules can tell us about populations: choosing and using molecular marker. Ecology, Vol. 79, PP: 361-382.

Pringle J. D., (1986). California spiny lobster (Panulirus interruptus) larval retention and recruitment: a review and synthesis. Can. J. Fish. Aquat. Sci. Vol. 43:2142-2152.

Pyle R. L., (1993). Marine Aquarium Fish. In: Nearshore Marine Resources of the South Pacific, A. Wright and L. Hill (eds). Institute of Pacific Studies, University of the South Pacific.

Ray N., Currat M., and ExcoYer L., (2003). Intra-deme molecular diversity in spatially expanding populations. Mol. Biol. Evol., Vol. 20:76– 86.

Roberts C.M., McClean C. J., Veron JEN, Hawkins J. P., R. A., McAllister D. E., Mittermeier C. G., (2002). Marine biodiversity hotspots and conservation priorities for tropical reefs. Science, Vol. 295: 1280-1284.

Rocha L. A., Bass A. L., Robertson D. R., Bowen B. W., (2002). Adult habitat preferences, larval dispersal, and the comparative phylogeography of three Atlantic surgeonWshes (Teleostei: Acanthuridae). Mol. Ecol., Vol. 11, 243–252.

Rogers A. L., and Harpending H., (1992). Population growth makes in the distribution of pairwise genetic waves differences. Mol. Biol. Evol., Vol. 9: 552–569.

Rogers A. R., (1995). Genetic evidence for a Pleistocene population expansion. Evolution, Vol. 49: 608-615.

Rousset, F., (1997). Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics, Vol. 145: 1219-1228.

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. Proc. Nat. Acad. Sci., Vol. 74 (12): 5463-7.

Schneider, S., Kueffer J. M., Roessli D., and Excoffier L. (2000). Arlequin. Ver. 2.0: software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Slatkin M., and Hudson R., (1991). Pairwise comparisons of DNA sequences in stable and exponentially growing populations. Genetics, Vol. 129:555–562.

Shulman M. J., and Bermingham E., (1995). Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution, Vol. 49(5): 897-910.

Sponaugle S., Cowen R. K., Shanks A., Morgan S.G., Leis J.M., Pineda JBoehlert G.W., Kingsford M.J., Lindeman K.C., Grimes C., and Munro J.L., (2002). Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. Bull. Mar. Sci., Vol. 70(1): Suppl.: 341- 375.

Stepien C. A., Randall J. E., and Rosenblatt R. H., (1994). Genetic and morphological divergence of a circumtropical complex of goatfishes: *Mulloidichthys vanicolensis*, *M. dentatus*, and *M. martinicus*. Pac. Sci., Vol. 48: 44–56.

Swearer S.E., Caselle J. E., Lea D. W., and Warner R.R., (1999). Larval retention and recruitment in an island population of a coral-reef fish. Nature, Vol. 402: 799-801.

Swearer S. E., Shima J. S., Hellberg M. E., Thorrold S. R., Jones G. P., Robertson D.R., Morgan S. G., Selkoe K. A., Ruiz G. M., and Warner R.R (2002). Evidence of self-recruitment in demersal marine populations. Bull. Mar. Sci., Vol. 70 (1): Suppl.:251-271.

Swofford D. L., (2002). PAUP*. Phylogenetic Analysis Using Parsimony. Version 4, Sinauer Associates, Sunderland Massachusetts.

Tajima F., (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics, Vol.125: 585–593.

Taylor M.S. and Hellberg M. E., (2003). Genetic evidence for local retention of pelagic larvae in a Caribbean reef fish. Science, Vol. 299: 107–109.

Taylor M. S., Hellberg M. E., (2005). Marine radiations at small geographic scales: speciation in neotropical reef gobies (Elacatinus). EvolutionVol. 59: 374–385.

Thresher R. E., (1984). Reproduction in Reef Fishes. Neptune City, NJ, T. F. H. Publishing.

Voris H. K., (2000). Maps of Pleistocene sea levels in Southeast Asia: shorelines, river systems and time durations. J. Biogeogr., Vol. 27: 1153−1167.

Wabnitz C., (2003). From Ocean to Aquarium: The Global Trade in Marine Ornamental Species. Published 2003 UNEP/Earthprint. 64 pages.

Wood E., (2001). Collection of Coral Reef Fish for Aquaria: Global Trade, Conservation Issues and Management Strategies. Marine Conservation Society, Vol. 3: 65–77, Ross-on-Wye, UK.

Williams, S. T. and Benzie J. A. H., (1997). Indo-West Pacific patterns of genetic differentiation in the high-dispersal starfish Linckia laevigata. Mol. Ecol., Vol. 6: 559–573.

Wyrtki K., (1961). Physical oceanography of the Southeast Asian waters. Naga Report, Scripps Institute of Oceanography, University of California, Vol. 2: 1−95.

2. Wichtigsten Positionen des zahlenmäßigen Nachweises

Finanzierungs-Plan

VA	bewilligt	1. Umwidmung	2. Umwidmung	3. Umwidmung
812	91.756,00	91.756,00	91.756,00	83.356,00
822	0,00	0,00	0,00	8.400,00
834	0,00	10.000,00	10.000,00	10.000,00
835	0,00	0,00	10.000,00	10.000,00
843	42.000,00	42.000,00	32.000,00	32.000,00
846	33.120,00	23.120,00	23.120,00	23.120,00
850	8.322,00	8.322,00	8.322,00	8.322,00
Gesamt	175.198,00	175.198,00	175.198,00	175.198,00

Ausgaben

VA	2004	2005	2006	2007	Gesamt	bewilligt	Rest
812	16.740,01	26.207,06	26.535,07	8.442,28	77.924,42	83.356,00	5.431,58
822	0,00	0,00	4.800,00	3.600,00	8.400,00	8.400,00	0,00
834	0,00	7.075,06	0,00	1.197,99	8.273,05	10.000,00	1.726,95
835	0,00	0,00	9.860,00	0,00	9.860,00	10.000,00	140,00
843	7.213,53	14.704,76	10.907,92	431,63	33.257,84	32.000,00	-1.257,84
846	8.381,96	10.602,41	2.900,91	7.275,87	29.161,15	23.120,00	-6.041,15
850	8.321,38	0,00	0,00	0,00	8.321,38	8.322,00	0,62
Gesamt	40.656,88	58.589,29	55.003,90	20.947,77	175.197,84	175.198,00	0,16

Einnahmen

VA	2004	2005	2006	2007	Gesamt
0000	43.936,00	55.388,00	55.844,00	20.030,00	175.198,00

HHJ	2004	2005	2006	2007
Kassenbestand	3.279,12	77,83	906,73	0,16

3. Notwendigkeit und Angemessenheit der geleisteten Arbeit

Die geleistete Arbeit war notwenig und angemessen. Es wurde zur Untersuchung der Konnektivität von Korallenrifforganismen, die im Aquarienhandel eine große Rolle spielen, insgesamt 8 Arten untersucht.

4. Voraussichtlicher Nutzen, insbesondere der Verwertbarkeit des Ergebnisses im Sinne des fortgeschriebenen Verwertungsplans

Es wurden keine Erfindungen/Schutzrechtsanmeldungen gemacht und solche sind auch in Zukunft nicht vorgesehen. Da es sich um ökologische Grundlagenforschung handelt, sind keine wirtschaftlichen Erfolgsaussichten nach Projektende zu erwarten.

Die Konnektivität von marinen Populationen spielt auch in der zweiten Projektphase eine Rolle, allerdings nur untergeordnet. Dennoch sind die im

Projekt erzielten Ergebnisse eine wichtige Grundlage für die wissenschaftliche Anschlussfähigkeit. Auf die gemachten Erfahrungen und bereits erarbeiteten Daten der ersten Phase wird daher aufgebaut.

5. Während der Durchführung des Vorhabens dem ZE bekannt gewordener Fortschritt auf dem Gebiet des Vorhabens bei anderen Stellen

Es sind keine Ergebnisse dritter Seite bekannt geworden, die für die Durchführung des Vorhabens relevant sind. Es wurden regelmäßig Informationsrecherchen in den Literaturdatenbanken BIOSIS und Web of Science durchgeführt. Außerdem wurden die jeweils neunen Ausgaben fachspezifischer Zeitschriften (z.B. Molecular Ecology, Marine Biology, Coral Reefs) gesichtet.

6. Erfolgten oder geplanten Veröffentlichungen der Ergebnisse

Die wissenschaftlichen Projektergebnisse sollen in wissenschaftlichen Fachzeitschriften publiziert werden. Zwei Manuskripte sind bereits eingereicht und and vier Manuskripten wird z.Z. gearbeitet (Details sieh unten). Die Sequenzdaten werden, zumindest zum Teil, in öffentlichen Internetdatenbanken (EMBL Sequence Database) zur Verfügung gestellt. Auf Grundlage der Projektergebnisse sollen Managementempfehlungen für die Aquarienfischerei ausgesprochen werden.

Publications

Submitted manuscripts

Nuryanto A, Kochzius M (2007) Strong genetic population structure in the boring giant clam *Tridacna crocea* across the Indo-Malay Archipelago. Molecular Ecology

Timm J, Figiel M, Kochzius M. Phylogeography and species boundaries of anemonefishes (Amphiprioninae, Pomacentridae) in the Indo-Malay Archipelago. Molecular Phylogenetics and Evolution

Manuscripts in preparation

Kochzius M, Hauschild J, Kirchhoff S, Meyer-Wachsmuth I, Seidel C (2007) Genetic population structure in the blue starfish *Linckia laevigata* and its parasite *Thyca crystallina* in the Indo-Malay Archipelago

Kochzius M, Krämer W, Jompa J, Borell E, Richter C (2007) A genetic survey on the diversity of zooxanthellae (*Symbiodinium* sp) in the scleractinian coral

Porites cylindrica along a pollution gradient in Spermonde Archipelago, Sulawesi, Indonesia

Kochzius M, Komalawati N, Nuryanto A (2007) Genetic diversity of 28S rDNA sequences in *Symbiodinium* from the giant clam *Tridacna* maxima across the Indo-West Pacific

Kochzius M, Wolter H (2007) Molecular systematics of lionfishes (Scorpaenidae, Pteroinae) and phylogeography of the *Pterois miles/P. volitans* species complex in the Indo-West Pacific

Nuryanto A, Kochzius M (2007) Genetic population structure of the giant clam *Tridacna maxima* (Tridacnidae, Bivalvia) in the Indo-West Pacific

Talks on conferences

Kochzius M (2006) Gene flow and connectivity of coral reefs across the Indonesian Archipelago: implications for the management of marine ornamentals. LOICZ-SPICE Conference, Bali, Indonesia

Nuryanto A, Kochzius M (2006) Limited gene flow in the giant clam Tridacna crocea across the Indo-Malay Archipelago. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Timm J, Kochzius M (2006) Strong genetic structure and limited gene flow between populations of the False Clown Anemonefish (*Amphiprion ocellaris*) in the Indo-Malay Archipelago. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Posters on conferences

Kochzius M, Timm J, Nuryanto A (2006) Genetic structure of coral reef animals across the Malay Archipelago: gene flow and evolutionary processes in the centre of marine biodiversity. Marine Genomics Conference, Sorrento, Italy

Kochzius M, Timm J (2006) Evolution of anemonefishes (Amphiprioninae, Pomacentridae). 1. Molecular phylogeny. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Timm J, Figiel M, Kochzius M (2006) Evolution of anemonefishes (Amphiprioninae, Pomacentridae). 2. Species boundaries in the Indo-Malay Archipelago. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Kochzius M, Hauschild J, Kirchhoff S, Meyer-Wachsmuth I, Seidel C (2006) Congruence of genetic population structure in the blue starfish *Linckia laevigata* and its parasite *Thyca crystallina* in the central Indo-Malay Archipelago? European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Kochzius M, Krämer W, Jompa J, Borell E, Richter C (2006) A genetic survey on the diversity of zooxanthellae (*Symbiodinium* sp) in the scleractinian coral *Porites cylindrica* along a pollution gradient in Spermonde Archipelago, Sulawesi, Indonesia. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Kochzius M, Komalawati N, Nuryanto A (2006) Genetic diversity of 28S rDNA sequences in *Symbiodinium* from the giant clam *Tridacna maxima* across the Indo-West Pacific. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Kochzius M, Wolter H (2006) Molecular systematics of lionfishes (Scorpaenidae, Pteroinae) and phylogeography of the *Pterois miles/P. volitans* species complex in the Indo-West Pacific. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Kochzius M, Froukh T, Timm J, Khalaf MA (2006) Molecular systematics of Indo-Pacific butterflyfishes (Chaetodontidae). European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

Mohammad S, Lange H, Krain E, Kochzius M, Jompa J (2006) Socioeconomic importance of marine ornamental fishery in Spermonde Archipelago, South Sulawesi, Indonesia. European Meeting of the International Society for Reef Studies (ISRS), Bremen, Germany

PhD theses

Agus Nuryanto (2007) Phylogenetic relationships and genetic population structure of giant clams (*Tridacna crocea* and *Tridacna maxima*) in Indonesia. IPB Bogor, Indonesia

Janne Timm (voraussichtlich Anfang 2008) Evolution and genetic population of clownfishes in the Indo-Malay Archipelago. University of Bremen

MSc theses

Mohammad S (2006) Socie-economic importance of marine ornamental fishery at Spermonde Archipelago, South Sulawesi – Indonesia. ISATEC, University of Bremen

Lemia MO Hamid (2007) Genetic population structure of the Pink Anemonefish, *Amphiprion perideraion* (Pomacentridae), in the Indo-Malay Archipelago. ISATEC, University of Bremen

Reports of undergraduate students

Meyer-Wachsmuth I (2006) Population structure in the parasitic snail *Thyca crystallina* in the central Indo-Malay Archipelago and a possible congruence to *Linckia laevigata*. University of Bremen

Seidel C (2006) Populationsgenetische Analysen der parasitären Schnecke *Thyca crystallina* im Indo-Malayischen Archipel. University of Bremen

Hauschild J (2006) Genetische Untersuchung des Seesterns *Linckia laevigata* auf Populationsstruktur im Indo-Malaysischen Archipel. University of Bremen

Kirchhoff S (2006) Untersuchung der populationsgenetischen Struktur des Seesterns *Linckia laevigata* im Indo-West pazifik. University of Bremen

Mester P (2006) Genetische Untersuchungen des Seesterns *Linckia laevigata* auf Populationsstruktur im Indo-Malayischen Archipel. University of Bremen

Zuwendungsempfänger: Alfred-Wegener-Institut für Polar- und Meeresforschung, Bremerhaven	Förderkennzeichen: 03F0390C
Vorhabensbezeichnung: **TP3.3 (UNHAS-AWI-Bremerhaven)** **Pelagische Prozesse und Plankton**	
Laufzeit des Vorhabens: Mai 2004 bis Juni 2007	
Berichtszeitraum: Schlussbericht	

Phyto- und Zooplankton sowie Schwebstoffe spielen eine wichtige Rolle als Nahrung für viele Organismen der Seegraswiesen sowie Rifforganismen, wie planktonfressende Fische und Korallen. Eine starke Erhöhung der Konzentrationen von Schwebpartikeln aufgrund von gesteigerten terrigenen Einträgen von Sedimenten, Nähr- und Schadstoffen führt aber zu einer Verschlechterung der Wasserqualität, da Korallen und Seegräser nur in klarem oligotrophem Wasser überleben können. Anthropogene Umweltveränderungen (z.B. Eutrophierung) werden als eine der Hauptgründe für die Zerstörung indonesischer Korallenriffe während der letzten Jahrzehnte angesehen. Trotz der großen Bedeutung von Schwebstoffen für die Lebensgemeinschaften Korallenriff und Seegraswiese ist das Vorkommen, die Konzentration und Zusammensetzung von gelöstem und partikulärem Material in der Wassersäule in indonesischen Küstengewässern wie dem Spermonde Archipel noch ebenso wenig bekannt wie die Reaktion von Phyto- und Zooplankton auf z.B. lokale Eutrophierung und Verringerung der Lichtintensität.

Das Ziel der pelagischen Untersuchungen während SPICE I war daher, den Einfluss verschiedener Umweltfaktoren (Temperatur, Salzgehalt, Nährstoffe) auf Schwebstoffe (Phyto- und Zooplankton, Seston) zu erfassen. Die quantitativen Untersuchungen wurden über verschiedene Raum- und Zeitskalen durchgeführt.

Großräumige Aufnahme

Für eine großräumige Untersuchung wurde das Plankton in Abhängigkeit von abiotischen Faktoren auf vier Transekten über das Schelfgebiet bis in den ozeanischen Bereich zum Ende der Regenzeit (März) und der Trockenzeit (September) untersucht. Die Konzentrationen der

abiotischen und biotischen Parameter änderten sich sehr entlang der Transekte von der Küste zur Schelfkante mit meist erhöhten Werten in Küstennähe aufgrund landseitiger Einträge und Flusseinleitungen.

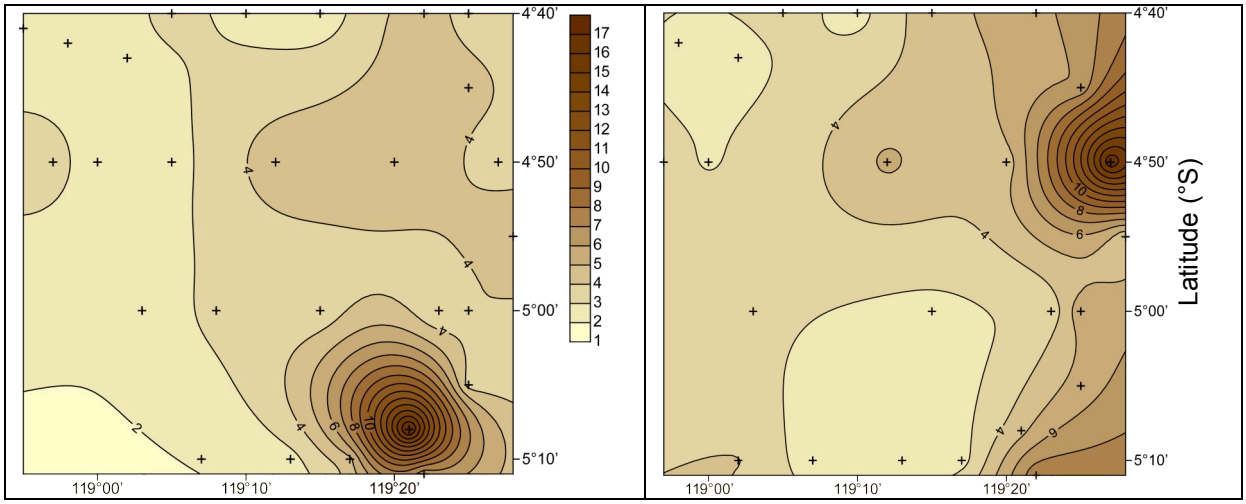


Abb. 1. Sestonkonzentrationen (mg L-1) in 3 m Tiefe (links) und in 20 m Tiefe (rechts).

Im September deuten kühleres und salzhaltigeres Wasser im Südwesten des Archipels auf ein lokales Auftriebsereignis hin. Dieses Auftriebsereignis ging einher mit erhöhten Nitratwerten. Unsere Ergebnisse weisen auffällig hohe Nährstoffkonzentrationen im Spermonde Archipel vor und stehen in Kontrast zu der Nährstoffarmut in tropischen Meeren. Der Grund hierfür ist sicherlich der Eintrag von terrigenem Material und ungeklärten Abwässern. Die Chlorophyll-Konzentrationen wiesen keine grossen Unterschiede zwischen beiden Untersuchungen auf. Von großer Bedeutung für bodennahe Prozesse sind sicherlich die gefundenen hohen Phytoplanktonbiomassen in den größeren Wassertiefen, die im März von Prymnesiophyten, Synechoccus, Pelagophyten und Diatomeen, im September dagegen bis auf wenige Stationen vorwiegend von Diatomeen gebildet wurden. Ein weiterer interessanter Unterschied ergab sich bei den Phaeopigmenten. Im März herrschte Phaeophytin in allen Wassertiefen vor, ein Hinweis auf abgestorbenes Phytoplankton. Im September dagegen wurden meistens Phaephorbide nachgewiesen, was auf hohe Grazing-Aktivitäten in der gesamten Wassersäule deutet.

Copepoden dominieren auf allen Stationen das Mesozooplankton, gefolgt von Appendicularien. Innerhalb des Meroplanktons dominieren die Larven von Echinodermaten in Küstennähe, während Larven der Gastropoden und Polychaeten eine größere Bedeutung auf küstenfernen Stationen zukommt. Insgesamt konnten 86 calanoide Copepodenarten identifiziert werden (Tab. 1).

Tab. 1. Liste der gefundenen Copepoden (*ozeanisch, **neritsch

Order Calanoida Sars, 1903
Family Calanidae Dana, 1849
*Canthocalanus pauper**
*Cosmocalanus darwini**
*Mesocalanus tenuicornis**
Nannocalanus minor
Neocalanus gracilis
*Undinula vulgaris**
Family Paracalanidae Giesbrecht, 1892
Acrocalanus gibber
A. gracilis
*A. longicornis**
*A. monachus***
*Bestiolina similis***
Calocalanus spp.
C. pavo
C. plumolosus
Delius nudus
Paracalanus spp.
*P. aculeatus**
P. denudatus
P. indicus
P. tropicus
*Parvocalanus crassirostris***
*P. scotti***
Family Mecynoceridae Andronov, 1973
*Mecynocera clausi**
Family Eucalanidae Giesbrecht, 1892
*Eucalanus inermis**
*Pareucalanus attenuatus**
*P. sewelli**
Rhincalanus rostifrons
*Subeucalanus crassus**
*S. mucronatus**
*S. pileatus**
*S. subcrassus**
Family Clausocalanidae Giesbrecht, 1892
*Clausocalanus arcuicornis**
C. farrani
*C. furcatus**
*C. mastigophorus**
C. minor
*C. parapergens**
C. paululus
Ctenocalanus vanus
Family Aetideidae Giesbrecht, 1892
*Aetideus acutus**
Family Euchaetidae Giesbrecht, 1892
*Euchaeta media**
E. wolfendeni
Family Phaennidae Sars, 1902
Xanthocalanus sp. *
Family Scolecitrichidae Giesbrecht, 1892
Scolecihtricella sp.
*S. beata**
S. pseudooculata
*Scolecthrix danae**

Family Diaixidae Sars, 1902
*Anawekia bilobata**
Family Tharybidae Sars, 1902
Tharybis sp. *
Family Mesaiokeratidae Matthews, 1961
Mesaiokera sp. *
Family Arietellidae Sars, 1902
*Metacalanus aurivilli***
Family Augaptilidae Sars, 1905
Haloptilus sp. *
Family Heterorhabdidae Sars, 1902
Heterorhabdus sp.
Family Lucicutiidae Sars, 1902
Lucicutia flavicornis
Family Metridinidae Sars, 1902
Pleuromamma sp.
Family Centropagidae Giesbrecht, 1892
*Centropages calanunus**.
*C. elongatus**
*C. furcatus**
Family Pseudodiaptomidae Sars, 1902
*Pseudodiaptomus aurivill***
*P. clevei***
Family Temoridae Giesbrecht, 1892
*Temora discaudata**
*T. stylifera**
*T. turbinata***
Temoropia sp.
Family Candaciidae Giesbrecht, 1892
*Candacia catula**
*C. curta**
*C. ethiopica**
*C. pachydactyla**
Family Pontellidae Dana, 1853
Calanopia aurivilli
*C. australica**
C. elliptica
C. herdmani
*C. minor**
*Labidocera acuta**
L. bengalensis
L. minuta
Pontellopsis herdmani
Family Acartiidae Sars, 1903
Acartia centrura
A. clausi
*A. danae**
*A. erythraea***
A. negligens
*A. pacifica***
Family Tortanidae Sars, 1902
Atortus sp.
Tortanus barbatus
T. gracilis

Order Cyclopoida Burmeister, 1934
Family Oithonidae Dana, 1853
Oithona spp.
Family Oncaeidae Giesbrecht, 1892
Oncaea spp.
Family Sapphirinidae Thorell, 1859
Sapphirina spp.
Copilia spp.
Family Corycaeidae Dana, 1852
Coryceaus spp.
Farranula spp

Order Harpacticoida Sars, 1903
Family Ectinosomatidae Sars, 1903
Microsetalla spp.
Family Miraciidae Dana, 1846
Macrosetella spp.
Miracia spp.
Family Euterpinidae Brian, 1921
Euterpina spp.
Family Clytemnestridae A. Scott, 1909
Cytemnestra spp.

Zwischen 21 und 48 Arten wurden auf den küstenfernen Stationen gefunden, zwischen 9 und 20 Arten in Küstennähe Die Familie Paracalanidae dominierte mit 70 bis 90% auf allen Stationen – allerdings unterschieden sich die Arten in ihrem maximalen Vorkommen (Abb. 2).

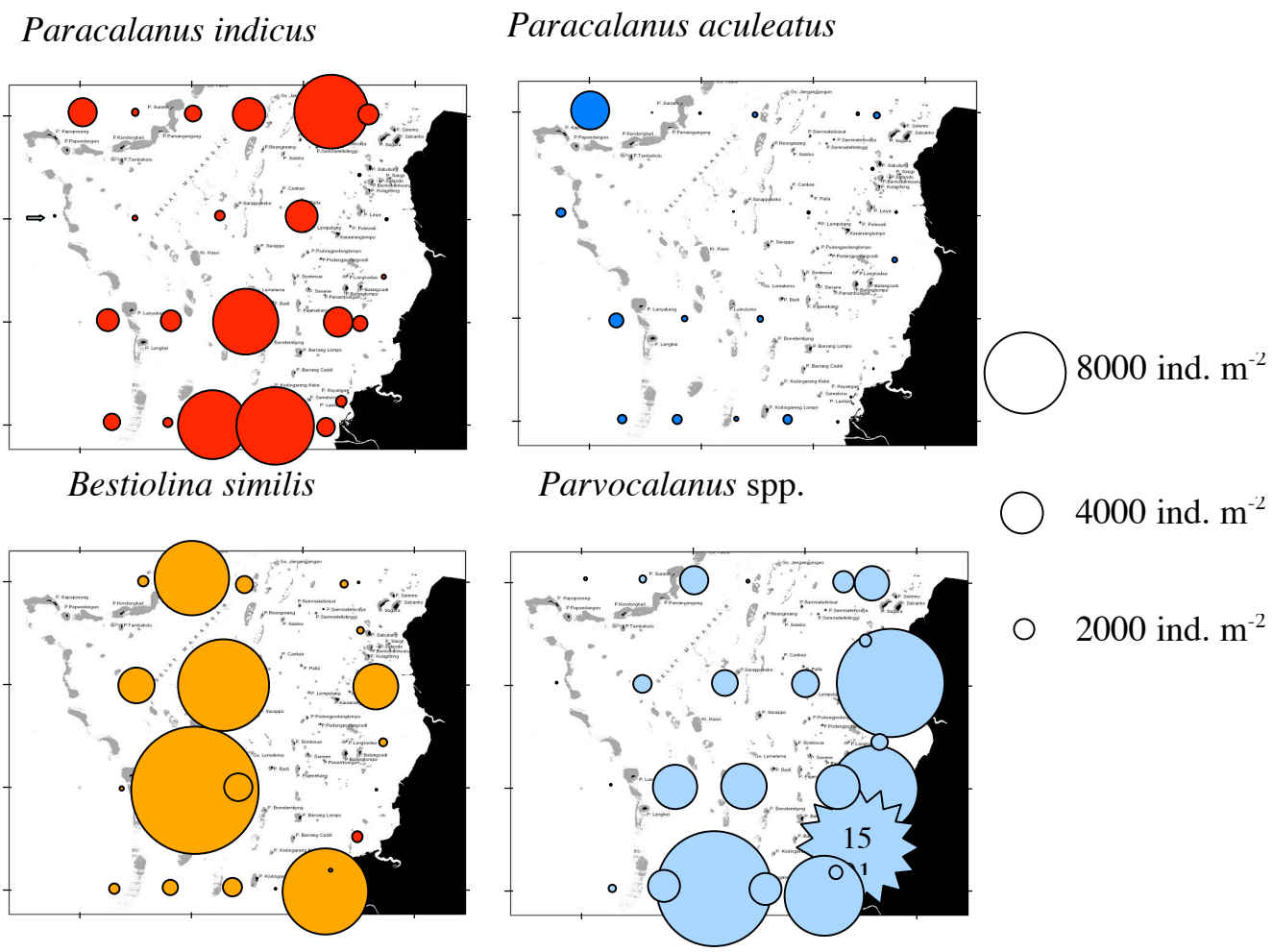


Abb. 2. Verbreitung von vier Paracalanidae Arten im Spermonde Archiopel

Die Ergebnisse der hydrographischen und planktologischen Parameter der Ausfahrten im März und September 2005 sind in eine Semesterarbeit (Bremer und Wilken 2005) und eine Diplomarbeit (Schmitt 2007) eingegangen. Die Bearbeitung der calanoiden Copepoden hat Frau Dr. Astrid Cornils, die innerhalb des internationalen Projektes Census of Marine Zooplankton als PostDoc im AWI eingestellt ist, übernommen. Die Ergebnisse wurden auf mehreren internationalen Tagungen vorgestellt (siehe Anhang), und zwei Publikationen (Umweltparameter und Phytoplankton, Copepoden) sind in Vorbereitung.

Kleinräumige Aufnahme und jahreszeitliche Untersuchungen

Zur Erfassung des jahreszeitlichen Verlaufs der planktologischen Parameter, wurden von August 2005 bis August 2006 kleinskalige Messungen an drei verschiedenen Riffen durchgeführt. Die drei beprobten Riffe lagen bei den Inseln Kayangan, Barang Lompo und Lanyukan, die sich aufgrund anthropogener Einträge (Sedimentation, Abwässer, Ölverschmutzung) sowie ozeanischer Einflüsse (Strömungen, Salzgehalt, Wellenbewegung, Wind, Monsum- Auswirkungen) sehr unterscheiden. Die Insel Kayangan liegt im Hafeneinzugsbereich von Makassar und wird durch den Industriehafen (z.B. Zementfabrik, Ölraffinerie) stark beeinflusst. Barang Lompo liegt auf dem mittleren Schelf noch relativ dicht bei Makassar. Die Bevölkerungsdichte beider Inseln ist hoch. Lanyukang, im äußeren Randgebiet des Spermonde Archipels nahe der Staße von Makassar, ist dagegen nur sehr dünn besiedelt. Dementsprechend unterscheiden sich die Inseln auch in der Trübung ihres umliegenden Wassers. Das Wasser um Kayangan ist stark, um Barang Lompo mittel getrübt und um Lanyukan ist es sehr klar.

Erhöhte Temperatur-, Salzgehalt-, Nährstoff- und Sestonkonzentrationen wurden auf den Stationen direkt über den Korallenriffen gemessen. Der Zooplanktonbestand war dagegen direkt über den Riffen am niedrigsten. Unterschiede in den Zooplanktonabundanzen gab es auch zwischen den drei Inseln und die höchsten Konzentrationen kamen auf den Stationen bei der küstennahen Insel Kayangan, die niedrigsten bei der küstenfernen Insel Lanyukan vor.

Meroplankton kam in höheren Abundanzen in Küstennähe vor. Insgesamt wurden 43 verschiedene Larvenformen gefunden (Tabelle 2). Die höchste Anzahl Larvenformen wurden auf Stationen bei der küstennahen Insel Kanyangan gefunden, die niedrigste bei der küstenfernen Insel Lanyukan. Mollusken und Polychaeten dominierten mit 70 bis 90% das Meroplankton. Plathelminthen, Echinodermaten, Crustaceen und Nemertinen waren weitere abundante Taxa.

Tab. 2. Liste der gefundenen Larvenformen (* nur bei Kanyangan, ** nur bei Barang Lompo).

- Porifera
  - Parenchymella**
- Cnidaria
  - Planula
  - Hydozoa
    - Actinula
  - Anthozoa
    - Cerinula
- Plathyhelminthes
  - Polycladida
    - Müllerlarva
  - Trematoda
    - Cercarie
- Trochozoa
  - Trochophora
  - Metatrochophora
- Mollusca
  - Gastropoda
    - Veliger
  - Bivalvia
    - Veliger
- Nemertini
  - Pilidium
- “Polychaeta”
  - Nectochaeta
  - late Nectochaeta
  - Rostraria
  - Terebellidae larvae
  - Polygordiidae
    - Endolarvae
  - Mageloniidae
    - Megelona
  - Oweniidae
    - Mitraria
- Crustacea
  - Cirripedia
    - Balanoidea Nauplius
    - Balanoidea Cypris
  - Facetotecta, Thecostraca
    - Orthonauplius
    - Cypris
- Malacostraca
  - Stomatopodalarva
  - Decapoda
    - Zoea
    - Megalopa*
    - other Decapoda laevae
- Phoronidea
  - Actinotrocha
- Inarticulata
  - Disciniacea
    - Brachiopoda larvae
  - Lingulacea
    - Brachiopoda larvae
- Bryozoa
  - Cyphonautes
- Echinodermata
  - Echinoidea
    - Echinopluteus
    - Echinopluteus metamorphosis
  - Asteroidea
    - Bipinnaria
    - Brachiolaria*
  - Ophiuroidea
    - Ophiopluteus
    - Ophiopluteus metamorphosis
  - Holothuroidea
    - Doliolaria
    - Pentactula**
    - Auricularia
- Sipunculida
  - Pelagosphaera**
- Enteropneusta
  - Tornaria
- Tunicata
  - Ascidiacea
    - Tadpole larvae
- Acrania
  - Branchiostoma

Die Untersuchungen zur kleinräumigen Verteilung fanden innerhalb einer Diplomarbeit statt (Schulz 2007). Frau Dipl.-Biol, Andrea Bäurle, die im SPICE Projekt als Doktorandin eingestellt war, hat den Jahresgang der meroplanktischen Larven an den drei verschiedenen Inseln im Spermonde Archipel untersucht. Frau Bäurle hat im Herbst 2007 eine Referendarausbildung begonnen, daher verzögert sich die Fertigstellung ihrer Dissertation bis zum Sommer 2008.

Examensarbeiten

Bäurle, A. (in Vorb.). Meroplankton larvae dirstribution off Makassar, South Sulawesi, Indonesia. Dissertation Universität Oldenburg

Bremer, K. & Wilken, S. (2006). Seasonal and spatial variation in phytoplankton composition and distribution in Spermonde Archipelago in South-West Sulawesi. Semesterarbeit Universität Kiel, 28 Seiten

Schmitt, P. (2007). Environmental influence on the distribution and community strcture of mesozooplankton in the Spermone Archipelago, Indonesia. Diplomarbeit Universität Bremen, 71 Seiten

Schulz, M. (2007). Zooplankton community structure in relation to environmental factors at Spermonde Archipelago, Indonesia. Diplomarbeit Universität Oldenburg, 120 Seiten

Vorträge und Poster auf internationalen Tagungen

38th International Liege Colloquium on Ocean Dynamics. Revisiting the role of Zooplankton in pelagic ecosystems. Liège, Belgium. May 2006

Cornils, A., Schnack-Schiel, S. B., Bäurle, A., Richter, C.: "Diversity of calanoid copepods off SW Sulawesi (Indonesia)“ (Vortrag)

Science Meets Policy for Coastal Management and Capacity Building, Bali, Indonesien, November 2006

Bäurle, A., Schnack-Schiel, S.B.: „Meroplankton larvae distribution off Makassar – preliminary results“ (Poster)

Cornils, A., Schnack-Schiel, S.B., Bäurle, A., Richter, C.: „Vertical distribution and diversity of calanoid copepods within the Spermonde Archipelago, SW Sulawesi, Indonesia“ (Poster)

Schnack-Schiel, S.B., Bremer, K., Wilken, S., Peeken, I., Kattner, G., Richter, C.: „Seasonal and spatial variation in phytoplankton distribution and composition in the Spermonde Archipelago in southwest Sulawesi, Indonesia“ (Vortrag)

Steering Group meeting of the Census of Marine Zooplankton (CMarZ), Tokyo, Japan, November 2006

Cornils, A., Schnack-Schiel, S. B.: "Zooplankton Research in Indonesia - SPICE" (Vortrag)

4th International Zooplankton Production Symposium: Human and climate forcing of zooplankton populations. Hiroshima, Japan, May 2007

Cornils, A., Schnack-Schiel, S. B., Bäurle, A., Richter, C.: "The impact of hydrographic parameters on the vertical and spatial distribution of calanoid copepod species on the shelf off SW Sulawesi (Indonesia)" (Vortrag)

TP3.2 (UNHAS-AWI Sylt):

Ökologie tropischer Seegraswiesen in indonesischen Küstengewässern

Förderkennzeichen 03F0390C

Abschlussbericht 2006

Zuwendungsempfänger: Alfred-Wegener-Institut für Polar- und Meeresforschung

Ausführende Stelle: Wattenmeerstation Sylt

Laufzeit des Vorhabens**:** Mai 2004 bis Juni 2007/ mit Verlängerung bis 31.7.2007

Berichtszeitraum: Mai 2004 bis Dezember 2006

Die Küstengebiete Indonesiens weisen weltweit die höchste Biodiversität in marinen Flachwasserarealen auf. Neben Korallenriffen und Mangroven haben besonders Seegraswiesen eine hohe ökologische Wertigkeit für den Naturhaushalt tropischer Küsten.
Daher wurde im Rahmen des interdisziplinären Forschungsprojekts SPICE (Science for the Protection of Indonesian Coastal Ecosystems) vom AWI aus ein deutsch-indonesisches Forschungsteam zur Erforschung der dort ausgedehnten Seegrasökosysteme etabliert.
Tropische Seegraswiesen gelten als Kinderstuben für zahlreiche kommerziell nutzbare Fischarten, und als Nahrungsgrund für seltene Meeressäuger (Dugongs) und Reptilien (Seeschildkröten). Sie tragen durch ihren dichten Bewuchs zum Schutz der Küsten und Inselstrände vor Erosion bei. Sowohl für angrenzende Mangroven- und Riffgebiete scheinen sie Funktionen auszuüben, die bisher noch nicht quantifiziert wurden. Erste Ergebnisse zeigen die Besiedlungsmuster der unterschiedlichen Seegrasassoziationen und Sukzessionsstadien für Makrofauna und Fische. Die Beobachtung experimentell eingeleiteter Sukzessionsfolgen ist geplant, um die Elastizität und die Wiederbesiedlung dieser Lebensgemeinschaften zu erfassen. Solche Untersuchungen können helfen, das natürliche Erholungspotential benthischer Lebensgemeinschaften nach Extremereignissen, wie etwa der jüngst erfolgten Tsunamiflutwelle in Westsumatra, zu ermitteln und vorherzusagen. Ziel der Untersuchungen 2004 bis 2007 war die quantitative Charakterisierung der Funktion der tropischen Seegraswiesen.

Das Ziel des Vorhabens war eine Bewertung des anthropogenen Einflusses (Algenfarmen, Fischerei) auf tropische Seegraswiesen und ihre assoziierte Fauna, insbesondere der Fischfauna riffgebundener, tropischer Seegraswiesen in Indonesien mit besonderer Berücksichtigung der nachhaltigen Entwicklung der umgebenden Küstenzonen und Korallenriffe.

Detaillierte Grundlagenforschung des Projektes wurde im Rahmen einer Doktorarbeit und 3 Diplomarbeiten durchgeführt. Darüber hinaus erbrachten Exkursionen, die zweimal im Jahr in den Spermonde Archipel führten, weitere Grundlagen zur Struktur und Funktion des

Ökosystems Seegraswiese. Die Exkursionen erbrachten Vergleiche zwischen Seegraswiesen unterschiedlicher Lage auf dem Schelf. Es wurden die wichtigsten Ökosystem- Kompartimente erfasst, wie die Biomasse und Abundanz der Primär- Sekundär und Tertiärkonsumenten des Systems. Im Gegensatz zu dem mehr beschreibenden Ansatz während der Exkursionen hatten die Diplomarbeiten experimentellen Charakter. Hier wurde die Rolle einzelner trophischer Gruppen oder Systemkompartimente für das System Seegraswiese gesondert untersucht. In der Doktorarbeit wurde versucht, eine Antwort auf spezielle sozio-ökonomische Fragen mit Hilfe von speziell auf diese Fragen zugeschnittenen Experimenten zu finden. Es wurden in dieser Weise insbesondere der Einfluss der expandierenden Algenfarmen und der lokalen Fischerei auf die Seegraswiese am Beispiel des kleinen Küstendorfes Puntondo bewertet.
Die Resultate gliedern sich in:

1. sozio-ökonomische Aspekte des Algenanbaus
2. Langzeiteffekte des Algenanbaus auf Seegraswiesen
3. Wirtschaftlich wichtige Fischarten, Fischereimethoden und Bedeutung bestimmter Küsten- Habitate, insbesondere Seegraswiesen für die Fischerei
4. Fischbestände und Kiemennetzfischerei in Seegraswiesen
5. Funktionell bedeutende Strukturelemente einer tropischen Seegraswiese

Auf Grund der Ergebnisse konnte die Nachhaltigkeit der Algenfarmen bewertet werden und die Frage beantwortet werden, ob diese Farmen eine alternative Existenzgrundflage zur Fischerei darstellen.
Die Ergebnisse des Projektes wurden in bisher 2 bereits akzeptierten peer- reviewed Publikationen verbreitet, vier weitere sind submitted und zwei weitere sind in Vorbereitung. Darüber hinaus wurden auf der internationalen Konferenz SPICE/LOICZ/ATSEF/SEACORM Konferenz, Science meets policy for coastal management and capacity building, sowie an Universitäten insbesondere in Asien vorgestellt.

1. Zusammenfassung der Ergebnisse der Dissertation:

Seaweed farming and artisanal fisheries in an Indonesian seagrass bed – Complementary or competitive usages ?

Küstendörfer in Indonesien waren schon immer stark auf traditionelle Fischerei angewiesen. Mit zunehmender Bevölkerungsdichte und Befischungsdruck werden alternative Einkommensquellen immer wichtiger, wovon eine, Algenanbau, seit den 1980ern in vielen Gemeinden eingeführt wurde. Algenanbau ist auf Flachwasser und seine Ökosysteme, z.B. Seegraswiesen, beschränkt, welche selbst ein wichtiger Lebensraum für viele Fisch-, Garnelen- und Krebsarten sind. Diese sind eine Grundlage der traditionellen Fischerei, und wenn Algenanbau Veränderungen im Seegrasökosystem verursacht, könnten seine ökonomischen Vorteile durch Einbussen in der Fischerei egalisiert werden. Die Feldforschung für diese Dissertation umfasste Untersuchungen zur Algenzucht, ihrer ökonomischen Bedeutung und Auswirkungen auf die Vegetation, zur Bedeutung traditioneller Fischereimethoden und deren Management und zur Varianz der Fischpopulation und der

Kiemennetzfischerei im Seegras vor Puntondo, einem kleinen Dorf im Kreis Takalar, Süd Sulawesi, Indonesien.
Kapitel 1 umfasst eine Studie zu ökonomischen Aktivitäten und dem für sie betriebenen Aufwand. Es wurden sowohl Probleme im Algenanbau und der Vermarktung der Ernte als auch im Fischereisektor bewertet. Beinahe alle Dorfbewohner bauten Algen an und ihr Haushaltseinkommen basierte zu einem Grossteil darauf. Sowohl Algenzucht als auch Fischerei wurde ein hohes Entwicklungspotential zugeschrieben und die Bewohner wollten beides ausbauen. Die Ergebnisse werden in Kapitel 1 detailliert diskutiert und aus ökonomischen Überlegungen heraus wird gefolgert, dass eine Intensivierung sich darauf konzentrieren sollte, die Produktivität durch eine bessere Ernteaufbereitung zu steigern. Eine Ausweitung in neue Gebiete sollte neben Überlegungen zu Standortfaktoren für die eigentliche Produktion auch den Zugang zu Märkten berücksichtigen, um den Dörfern ein kontinuierliches und vorhersagbares Einkommen zu ermöglichen. Darüber hinaus sollten auch andere Einkommensquellen bedacht werden, da der Preis für Algen sehr stark schwanken kann.

In Kapitel 2 wird der Einfluss von Schattierung, Tritt, einer Kombination aus beidem und von Algenfarmen auf Seegras betrachtet. Starke Beschattung verminderte die Wüchsigkeit von kleinen Seegrasarten, wohingegen Tritt nur in Verbindung mit Beschattung Auswirkungen hatte. Im Vergleich dazu wurde die große Seegras- Art *Enhalus acoroides* weit weniger beeinflusst. Die Auswirkungen von Algenanbau selbst waren geringer als aufgrund der Beschattungs- und Trittmanipulationen erwartet werden konnte.
Die Ergebnisse werden in Kapitel 2 ausführlich besprochen und eine Algendichte von ungefähr 185.000 Pflanzen ha^{-1} wird als nachhaltig abgeschätzt. Tatsächliche Anbaudichten auf Farmen waren unter diesem Grenzwert. Dennoch sollte eine Produktionssteigerung aus ökologischer Sicht durch neue Farmen in tieferem Wasser mit Abstand zu fragilen Systemen (Korallenriffe und Seegraswiesen) erfolgen. Dort ist eine höhere Produktion als auf Riffdächern zu erwarten und die Mehreinnahmen können die hohen Investitionen für geeignete Anbaumethoden ausgleichen.

In Kapitel 3 sind lokale Fischereimethoden, Fischgründe, Zielarten und deren Biologie der Schwerpunkt. Fischanlandungen wurden von einigen wenigen Methoden und Fischgründen dominiert, von denen die meisten nicht nachhaltig genutzt wurden. Seegraswiesen waren weder bedeutendes Habitat für wichtige Fischarten noch bedeutende Fischgründe. In Kapitel 3 wird angeraten, dass ökonomisch unbedeutende, aber nicht nachhaltige Methoden in sensitiven Fischgründen eingestellt werden sollten. Kiemennetze waren das wichtigste Fanggerät und Beschränkungen der Maschenweite könnten ihre Umweltverträglichkeit verbessern. Fischarten aus dem Riffbereich waren tendenziell überfischt und daher wird dort zu generellen Schonzeiten und / oder Beschränkungen der Methoden geraten. Im Allgemeinen waren mit Handangeln gefangene Fische relativ adult und diese Methode wird daher besonders empfohlen. Senknetze können ebenfalls umweltfreundlich angewandt werden, wenn untermassige Fische unmittelbar nach dem Fang wieder freigelassen werden.

In Kapitel 4 wird die Kiemennetzfischerei in der Seegraswiese und ihre Schwankungen behandelt. Generell wurden hauptsächlich kleine Fische aus nur wenigen Familien gefangen. Der Anteil adulter Tiere am Fang unterschied sich nicht zwischen verschiedenen Maschenweiten. Ein hoher Anteil von Räubern stellt die Funktion des Seegrases als Kinderstube in Frage. Räuber schwammen tagsüber in die Wiese ein, und in den Tagen um Vollmond und während der Trockenzeit war der Anteil adulter Tiere höher. Kleine Individuen wurden nicht gefangen und eine Räuber-Beute Beziehung konnte nicht als entscheidend für diese Schwankungen festgestellt werden. Die Ergebnisse lassen nicht auf eine Nützlichkeit von generellen Schonzeiten oder Beschränkungen von Maschenweiten zum Bestandsschutz der Fische im Seegras schließen. In Kapitel 4 wird angeraten, dass stattdessen die Biologie ökonomisch wichtiger und sensitiver Arten die Grundlage für eine Bewirtschaftung sein sollte.

Die gegenwärtigen Nutzungen – Algenanbau und Fischerei – beeinflussen sich gegenseitig nicht nennenswert. Die praktizierten Anbaumethoden schädigen die Vegetation kaum und die Qualität des Lebensraumes Seegraswiese wird nur minimal beeinträchtigt. In Hinsicht auf die geringe Bedeutung der Fischerei im Seegras bzw. auf Arten, die von ihm abhängig sind, werden schwache Veränderungen im Seegras die traditionelle Fischerei als ganzes nicht beeinflussen. Beim Ausbau des Algenanbaus in Zukunft sollte jedoch darauf geachtet werden, das Seegrassystem nicht über seine Toleranzschwelle hinaus zu belasten. Ohne angepasste Methoden wäre diese bald erreicht. Off-shore Algenfarmen könnten eine Lösung mit Synergieeffekt mit der Fischerei sein. Pelagische Schwarmfische sammeln sich unter schwimmenden Strukturen an und können dort gezielt befischt werden. Viele traditionelle Fangmethoden überfischen die Bestände eindeutig und die meisten Habitate sind überfischt. Die Produktivität kann nicht mit einem höheren Aufwand gesteigert werden. Es gibt experimentelle Fischfarmen im Dorf und ihre Weiterentwicklung in Hinsicht auf lokale Anwendbarkeit wäre nützlich, vorausgesetzt dass ihr Einfluss auf die Umwelt abgeschätzt wird.

1. Seaweed farming in traditional fishing villages: An example from South Sulawesi, Indonesia
(submitted to Ocean and Coastal Management)

Abstract

Seaweed farming has been introduced in many coastal communities in Indonesia as a means of improving local livelihood. In this study, its impact on the economy of a small village in South Sulawesi, Indonesia, was investigated. Similar to experiences from other areas, seaweed farming was the main source of income for most families in the village, though artisanal fisheries still played an important role. Locals had high hopes for the future for all economic activities, though problems may arise in the future. Especially area conflicts between seaweed farming and fisheries, though not rated important during the study, have the potential to limit sustainable development.

2. Long-term effects of floating long-line seaweed farming on seagrass shoot density and biomass
(submitted to Marine Ecology Progress Series)

Abstract

In a seagrass bed at the coast of South Sulawesi (Indonesia), the impact of seaweed farming has been investigated by simulating special processes occurring in cultivation plots where macroalgae are cultivated on floating ropes. Among the different possible influnces, shading and trampling were assumed to have the most significant effects on natural seagrass beds. The combination of different levels of both impacts was used to simulate different farming intensities on experimental plots. Additionally, on seaweed farms different algae seedling densities were applied. On all test fields, changes of seagrass shoot density and biomass were recorded over a period of two years. Shading has proved to be the strongest effect on seagrass shoot density and biomass development. Although seasonal species-specific dynamics determined density and biomass patterns of seagrasses, high shading levels reduced shoot density and biomass generally, whereas trampling was effective in combination with medium and high shading only. Actual farming was less damaging than could be expected from shading and trampling manipulations. The results of the study were used to estimate threshold levels for a maximum sustainable standing crop of seaweeds. At approximately 205.000 and 185.000 seedlings ha–1 for the community of smaller species (mainly Cymodocea serrulata and Thalassia hemprichii) and Enhalus acoroides, respectively, a rotating farming scheme does not influence seagrass significantly. Other

functional groups of the seagrass ecosystem (e.g. macrozoobenthos and fishes) were not included in this study and their tolerance levels towards seaweed farming might be lower.

3. Fishermen‘s perception of artisanal fisheries and implications for management in a bay in South Sulawesi, Indonesia
(submitted to Ocean & Coastal Management)

Abstract

Fishermen in Puntondo, a small village in South Sulawesi, Indonesia, were interviewed about their perception of current fisheries status and its development in recent years. They were asked for target species, individual size of fishes as well as abundance and its change. Also information on fishing habitats, on fishing methods, such as used gear, and market prices were collected. From literature, the data-set was completed with information on trophic levels, maximum sizes and sizes at first maturity of the fishes. Nets and lines were the most important fishing methods. Nets were more efficient to catch smaller size classes of fish compared to lines and tended to over-exploit fish stocks whereas lines caught relatively large fish.
Coral reefs and beaches were the most important fishing grounds. On both, catch abundance had decreased and fishes were comparatively small. For the whole bay however, overall fish abundance had not decreased significantly. Abundance change of individual species was negatively correlated with trophic level, indicating substantial fishing pressure in the area. Based on the status of fisheries perceived by the local people different methods and fishing habitats are evaluated in respect to improving fisheries management.

4. Variations in fish stocks and artisanal gill net fishery in an Indonesian seagrass bed
(submitted to Fisheries Management and Ecology)

Abstract

Seagrass beds are widely exploited by fishers in an Indonesian village in South Sulawesi, Indonesia, commonly using gill nets. This technique was also used to catch fishes to estimate the seasonality of abundance and species composition, and the influence of gill net fishery on local fish stocks. The results of this study indicate that gill net fishery covers a wide spectrum of species that is dominated by piscivorous fish families. Small mesh sizes were most effective. Seasonal variations were more dominant than diurnal or lunar variations, but year to-year variation was high.

2. Ergebnisse aus den Diplomarbeiten:

In den Diplomarbeiten wurden einzelne funktionell bedeutende Strukturelemente einer tropischen Seegraswiese herausgehoben und ihre Rolle für das Gesamtsystem experimentell ermittelt.
Die 1. Diplomarbeit beschäftigt sich mit der Rolle wirbelloser Weidegänger, die meist die reichlich vorhandenen Epiphyten auf den Seegrasblättern nutzen. In den Seegraswiesen der Insel Sulawesi spielen besonders synaptide Holothurien eine wichtige Rolle als Weidegänger. Ihre Rolle konnte in dieser Arbeit zum ersten Mal quantifiziert werden. Ein Effekt dieser Tiergruppe auf den Epiphytenbewuchs war jedoch bei natürlicher Dichte nicht statistisch abzusichern, obwohl die Tendenz deutlich nachweisbar war. Vermutlich wird durch die Klebtentakeln nur das leicht umsetzbare organische Material von den Seegurken aufgenommen, wegen der hohen anorganischen Anteile der Epiphyten (vermutlich Kalk durch Kalkalgen), das den größten Anteil am Gesamtepiphytengewicht hat, aber offenbar nicht mitgefressen wird, sind daher die Unterschiede am Gesamtepiphytengewicht durch Wegfraß eher gering. Bestimmungen des aschefreien Trockengewichtes waren vor Ort leider nicht durchführbar.

Die 2. Diplomarbeit beschäftigt sich mit den Verwertern des in einer Seegraswiese reichlich anfallenden Detritus. Über diesen Weg wird der Grossteil der Primärproduktion einer Seegraswiese genutzt. Die Untersuchungen hierzu konzentrierten sich auf die Barang Lompo benachbarte Sandbank Bonebatang. Hier wurden in Zusammenarbeit mit einer niederländischen Arbeitsgruppe von der Universität Nijmegen Untersuchungen zur mit Seegras assoziierten Endofauna durchgeführt.
Es wurde die Rolle der thalassiniden Decapoden der Gattung *Neaxius* und *Corallianassa* untersucht. Beide Arten spielen eine aktive Rolle bei dem Abbau von Seegras, indem sie treibende Seegrasblätter einfangen und in besondere Kammern in ihren bis zu 1m tiefen (*Corallianassa*) und 4 l Volumen (*Neaxius*) fassenden Bauten einlagern oder aber die Wände der Röhren zur Stabilisierung damit tapezieren (Abb. 4). Mit Seegras vermischtes Sediment wird in erweiterten Kammern ständig durch den Krebs durchgewühlt, vermutlich zu Fraßzwecken. Meist werden die Bauten paarweise bewohnt. Verhaltensexperimente von *Neaxius acanthus* in Aquarien wurden in der Station Barang Lompo durchgeführt. Hier konnte eine starke Territorialität der Krebse untereinander nachgewiesen werden. Außerdem konnten Angaben zum Aktivitätsrhythmus gewonnen werden. Bei der Untersuchung der Krebsbauten konnte eine besondere “underground community” beschrieben werden, die die Bauten dieser Krebsarten bewohnt.
Zu dieser Lebensgemeinschaft zählt eine kleine Grundel, *Austrolethops wardi*, die zum ersten Mal sowohl als Mitbewohner von Krebshöhlen als auch für Indonesien nachgewiesen werden konnte. Diese Fischart wurde ebenfalls hauptsächlich paarweise angetroffen. Weiterhin besteht die Lebensgemeinschaft aus Bivalviern, Polychaeten und Amphipoden, die ausschließlich innerhalb der Bauten angetroffen wurden.
Messungen der Nährstoffkonzentrationen in den Krebsbauten erbrachten gegenüber dem Außenmedium um ein Vielfaches erhöhte Ammonium- und Phosphatkonzentrationen. Bei einer durchschnittlichen Dichte von 2 Bauten pro m², ist daher mit einem deutlichen Einfluss dieser Systeme auf den Nährstoffhaushalts einer Seegraswiese zu rechnen.

Im Jahre 2006 wurde eine Diplomarbeit begonnen, in der insbesondere die Gobiidenfauna der Seegraswiesen im Vordergrund stand. Von den weltweit 2000 Arten der Grundeln kommen 272 Arten in Indonesien vor. Diese Fischgruppe ist von großer Bedeutung als Nahrung für größere Raubfische. In den Tropen zeigt diese Gruppe eine hohe Spezialisierung auf bestimmte Biotope und kann daher Lebensräume charakterisieren. Alle in einer Seegraswiese bei Baranglompo gefundenen Grundelarten lebten in Symbiose mit Garnelen, wobei der Fisch meist den Eingang bewacht und der Krebs mit dem Ausbau der Höhlungen beschäftigt ist, was deutlich zur Bioturbation und zum Remineralisierungsprozess einer Seegraswiese beiträgt. Es konnten 7 Arten in der Seegraswiese nachgewiesen

werden, unter denen die Art *Cryptocentrus caerulomaculatus* die häufigste war. Von der Gattung *Cryptocentrus* wurden 5 weitere Arten gefunden, die insgesamt aber nur 22% an der Gesamtabundanz ausmachten. Die Gesamtindividuenzahl an Gobiiden war negativ mit der Seegrasdichte korrelliert, da die genannten Arten an Krebsbauten gebunden sind, die vermutlich das relativ feinkörnige Seegrassediment meiden. Eine Ausnahme ist aber die Grundel *Austrolethops wardi*, die in den Bauten des seegrasfressenden Krebse *Neaxius acanthus* lebt. Diese Art war in den Seegraswiesen häufiger als an dessen Rand. Nahrungsuntersuchungen durch Mageninhaltsanalysen wiesen darauf hin, daß diese Grundel vermutlich eine herbivore Lebensweise hat und das Seegras selbst als Nahrungsquelle nutzt. Daher ist dieser Fisch in das kleine Nahrungsnetz der Krebsbauten eingegliedert und ist ein Einzelfall in der Nahrungsbiologie der Gobiiden.

1. The effect of synaptid holothurians (Synaptidae) on epiphyte biomass and macrophyte growth of the tropical seagrass *Enhalus acoroides* in Indonesian seagrass beds

Seagrasses are marine angiosperms which form extensive meadows on predominately sandy inter- and subtidal habitats throughout the world. They are known to have a variety of functions including sediment stabilization, sediment trapping, nutrient recycling, and nursery/habitat for economically valuable fish and invertebrate species. Furthermore, they provide a firm substratum which is used by a huge array of epiphytic algae and other periphyton, which contribute substantially to the primary production of a seagrass bed. These epiphytes are being grazed on by a variety of gastropods, crustaceans and some fish. Exclusion experiments showed that these grazers have a negative effect on epiphyte biomass and a positive effect on macrophyte growth. The knowledge about the interactions of seagrasses with their epiphytes and grazers are unequally distributed, with the centre of information being in temperate regions, the tropical West Atlantic and around Australia. Studies in the tropical Indo Pacific are scarce, and specifically in the world's largest archipelagic state Indonesia, grazer-epiphyte interactions have as of yet been investigated quantitatively.

This study's aim was the identification of potential grazers in seagrass beds around the south-west coast of Sulawesi, located in eastern Indonesia. Their effect on epiphyte biomass and macrophyte growth was investigated by the means of cage exclusion experiments. Preliminary surveys revealed two species of synaptid holothurians, *Opheodesoma sp*. and *Euapta sp*., as prominent members of the mixed intertidal seagrass beds in the Puntondo bay. A directed feeding behaviour was observed, such as the synaptids use the adhesive nature of their skin to crawl along the leaves of the biggest seagrass species *Enhalus acoroides*, in order to systematically place their tentacles on the leaf surface. By doing so, they were assumed to scrape off organic material from the leaf surface, which is then transferred to the mouth for ingestion. In order to test the effect of this feeding behaviour on the epiphyte biomass and macrophyte growth of *Enhalus acoroides*, cages were deployed in the field during wet and dry season. Treatments were then set up where the grazers were present and where grazers were excluded. Furthermore, open cages were set up as a control for cage artefacts as were experimental plots without cages. It was hypothesised that the presence of grazers would decrease the epiphyte biomass compared to the treatments without grazers, and that the macrophyte growth would be increased accordingly.

Differences were found when all treatments were pooled according to the two seasons. Epiphyte and macrophyte parameters increased in the wet season, while ANCOVA revealed significance only for the macrophyte growth. The increased productivity during the wet season was also reflected in increased temperature, dissolved oxygen, and pH, especially during low tide. When the measurements were taken during the dry season on the central leaf of the shoot only, macrophyte growth was only found to be significantly higher in the Open Cage treatment compared to the No Cage treatment. The other treatments and epiphyte biomass did not show significant differences and only a very weak correlation between epiphyte biomass and macrophyte growth was found. In the wet season, no

significant differences were found for the data on the central leaf, but the epiphyte and macrophyte values showed a higher correlation. The lowest epiphyte biomass in the *Euapta* treatment resulted in the highest macrophyte growth. When the peripheral leaves were incorporated into the measurements, significant differences were found to exist between the No Cage treatment with the highest epiphytic load and the *Euapta* and Open Cage treatment, with the lowest epiphytic load. The macrophyte growth did not differ significantly between the treatments when the peripheral leaves were measured.
Although only few statistically significant differences could be detected, a trend is still visible that the presence of the bigger synaptid *Euapta sp*. nevertheless reduces epiphyte biomass and thus results in higher macrophyte growth values. The smaller synaptid *Opheodesoma sp*. constantly results in similar epiphyte biomass values to the No Grazer treatment, but the macrophyte growth is still elevated. The minor efficiency of the smaller feeding apparatus compared to the bigger *Euapta sp*., results in the removal of smallest particles from the seagrass leaf. This cannot be detected by the means of scraping off the epiphytes and weighing them, but it still has a beneficial effect on the macrophyte through the increase of light and nutrient availability. This is the first study to quantitatively describe synaptid holothurians as epiphyte grazers on seagrass leaves, even though the effect is less pronounced than expected.

2. The role of *Neaxius acanthus* (Thalassinidea: Strahlaxiidae) and its burrows in a tropical seagrass meadow, with some remarks on *Corallianassa coutierei* (Thalassinidea: Callianassidae)
(parts of this Diploma thesis are published in Journal of Fish Biology (in press) and in Estuarine, Coastal and Shelf Science (accepted))

Diese Arbeit stellt einen Versuch dar, den Einfluss einer Population von *Neaxius acanthus*, einem Driftmaterial fangenden grabenden Krebs auf sein Habitat, eine tropische Seegraswiese zu beschreiben.
Durch die Überwachung von Bautenöffnungen in permanenten Transekten von September 2005 bis Januar 2006 konnte gezeigt werden, dass die Anzahl der Bautenöffnungen in einem Gebiet mit dichter Seegrasbedeckung größer ist als in einem Gebiet mit spärlicher Bedeckung. Der durchschnittliche Öffnungsdurchmesser wurde im September nicht erfasst, blieb aber von Oktober (18 mm) bis Januar (18 mm) unverändert. Mindestens 75% der Bauten werden von Paaren bewohnt. Die Öffnungen waren zu 65% offen, ansonsten waren sie verschlossen oder durch Sedimentauswurfshügel ersetzt. Der Bau von *N. acanthus* besteht aus einem senkrechten bis schrägen Schaft, der nach unten in eine große, mit Kies und Seegras gefüllte Kammer mündet. Alle Wände mit einer Steigung von mehr als 45° sind mit einer dicken braunen Auskleidung aus kleinen Sedimentkörnern und Bruchstücken mehrzelliger Pflanzen bedeckt. Das Auftreten der Grundel *Austrolethops wardi* als Kommensale von *N. acanthus* wird das erste Mal beschrieben. Weiterhin wurden eine Muschelart, zwei Arten von röhrenbildenden Polychaeten und eine Amphipodenart als Kommensalen gefunden. Eine palaemonide Garnele kann ebenfalls regelmässig in der Bautenöffnung gefunden werden. Bei Niedrigwasser suchen kleine Krabbenarten und juvenile Fische der Gattungen *Dischistodus* und *Apogon* in der Bautenöffnung, welche dann einen kleinen Gezeitentümpel darstellt, Schutz. Der Bau von *Corallianassa coutierei*, einem mit *N. acanthus* koexistierenden Driftmaterial fangenden Callianassiden, gleicht einem tiefen U. Eine der beiden Öffnungen ist immer nichtfunktional und mit Sediment verschlossen. Auf halber Höhe des funktionalen Schaftes liegt eine mit Kies gefüllte Kammer, am tiefsten Punkt zweigen mehrere mit Kies und Seegras gefüllte Kammern ab. Die Wände sind mit einer dünnen Auskleidung aus feinen Sedimentkörnern bedeckt. Wassergehalt, organischer Gehalt und die Schlickfraktion sind größer in den Bautenauskleidungen beider Arten als im umgebenden Sediment. Dieser Effekt ist deutlicher für *N. acanthus*. Messungen der Nährstoffgehalte (Ammonium, Phosphat) in der Wassersäule, im Porenwasser des Sediments in Tiefen von 1,5 bis 45 cm und in Bauten von *N. acanthus* zeigten, dass die Ammoniumkonzentration in den Bauten mit der des Sediments in einer Tiefe von 45 cm vergleichbar ist. Die Konzentration an reaktivem Phosphat in den Bauten ist höher als

anderswo. Die für Sauerstoff und pH gemessenen Werte liegen zwischen denjenigen für Sediment und Wassersäule. Individuen von *N. acanthus* zeigen einen Sexualdimorphismus der grösseren Schere. Es gibt eine Korrelation zwischen Bautendurchmesser und Carapaxlänge. Die Korrelation zwischen Carapaxlänge und Nassgewicht, Trockengewicht und aschefreiem Trockengewicht ist für Männchen, eiertragende Weibchen und Weibchen ohne Eier verschieden.
Das Verhalten der Tiere im Bauteneingang wurde *in-situ* beobachtet. Der Eingang wurde immer von einem der Bewohner „bewacht". Durchschnittlich 1,920 g Trockengewicht Öffnung^{-1} d^{-1} an Blattdetritus wurden von den beobachteten Krebsen gefangen. Das entspricht mehr als 50% der Primärproduktion in diesem Gebiet. Bei der Beobachtung des Verhaltens von Tieren in schmalen sedimentgefüllten Aquarien konnte festgestellt werden, dass während der Konstruktion und Instandhaltung des Baues nur wenig Sediment an die Oberfläche gebracht wird. Kleine Körner werden aus Sediment, welches mit den vorderen Fortsätzen aufgewirbelt wird, ausgesiebt. Diese Körner werden dann mit einer klebrigen Substanz, die von den Tieren ausgeschieden wird, vermischt und in die Wand gestopft. Zusätzlich wird feines Sediment in der Umgebung der unteren Kammer abgebaut. Durch die Definition von fünf Verhaltensklassen in denen 12 Verhaltenzustände enthalten sind wurde gezeigt, dass die Tiere die meiste Zeit mit Konstruktion verbringen, gefolgt von Inaktivität, Fortbewegung, Putzen und Fressen. Es wurde kein Unterschied in der Tag- und Nachtaktivität gefunden. Angebotenen Seegrasblätter wurden zerrissen und später in die Wand eingearbeitet.
Die Krebse verhalten sich höchst aggressiv gegenüber Geschlechtsgenossen, neue Baubewohner des anderen Geschlechtes werden aber sofort akzeptiert. Um Bautenöffnungen von *N. acanthus* herum wurde eine Abnahme von Seegrassprossdichte und Seegrasbiomasse um etwa 50% festgestellt. Durch den Einsatz von Detritusfallen wurde der passive Eintrag von Detritus in Bauten von *N. acanthus* auf etwa 1,386 g Trockengewicht Öffnung-1 d-1 geschätzt. Die Aktivität der Krebse erhöht den Eintrag demnach um 39%.

Zusätzlich zu den Untersuchungen in der genannten Diplomarbeit konnte das Nahrungsnetz des Bautensystems der thalassiniden Krebse mit Hilfe stabiler Isotopen und mit Hilfe von Mageninhaltsuntersuchungen aufgeschlüsselt werden.:

A seagrass based food web formed by the mud shrimp *Neaxius acanthus* (Thalassinidea: Strahlaxiidae) and the commensal community in its burrows.
(prepared for Ecology)

The food sources of Neaxius acanthus and associated macrobenthic animals living in its burrows have been analysed. The predominant food item of all animals which were big enough to allow a dissection of guts (*N. acanthus*, *C. coutierei* and *A. wardi*) was seagrass. Senescent detached leaves rather than fresh ones are assumed to be available for the species observed.
A box is therefore plotted, defined to the left by δ13C = mean of senescent *Halodule* – 1SD, to the top by δ15N = mean of senescent *Halodule* + 1SD, to the right by δ13C = mean of senescent *Thalassia* + 1SD and to the bottom by δ15N = mean of senescent *Thalassia* – 1SD (Fig. 1). Interestingly, this box contains the means measured for the burrow lining of both *Neaxius* and *Corallianassa* and the values for leaf litter of all seagrass species (Fig. 1).
Assuming an isotopic diet-tissue fractionation (Δ) of 0-1‰ for δ13C and 3-4‰ for δ15N (SOURCE) a plot was created: the slope of line α is parallel to the y-axis (Δδ13C = 0‰, Δδ15N = 4‰, the biggest values commonly accepted, respectively) and the slope of line β is 3 (Δδ13C = 1‰, Δδ15N = 3‰, the smallest values commonly accepted, respectively). Line α starts at the point (mean δ13C of old *Halodule* leaves + 1SD, mean δ15N of old *Halodule* leaves + 1SD) and line β starts at the point (mean δ13C of old *Thalassia* leaves - 1SD, mean δ15N of old *Thalassia* leaves - 1SD) (Fig. 1).
The trophic levels of the animals can then be estimated from then commonly accepted range of Δδ15N = 3-4‰, with a line a (δ15N = mean of old *Thalassia* – 1SD + 3 = 4.35) and a line b (δ15N = mean of old *Halodule* + 1SD + 4 = 6.98) (Fig. 1).

All species except for the Amphipods and the bivalve can therefore be considered primary consumers of senescent seagrass leaves, since their means are positioned within the area A defined by α, β, a and b.
It has to be considered that the treatment of the Amphipods has been different to the treatment of the other animal samples due to their small size (acidified whole animals vs. non-acidified muscle tissue). Yokoyama et al. (2005) state that Δδ13C and Δδ15N can vary within one species depending on the treatment; their values measured for acidified whole animals were smaller than for untreated muscle tissue. Literature values for the diet-tissue fractionation range from -1.5 to -0.4 (C) and -0.7 to 2.7 (N) for whole amphipods treated with HCl (Macko et al. 1982). Amphipods are commonly considered to be important consumers of seagrass detritus.
While an isotopic fractionation (Δ) for δ13C of 0-1‰ and Δδ15N of 3-4‰ is commonly accepted in the literature, the values for decapod muscle tissue not treated with HCl range from 1.1 to 3.1 (C) (Stephenson et al. 1986) and 2.4 to 3.7 (N) (Parker et al. 1989, Yokoyama et al. 2005). In Gaston & Suthers (2004), the diet-tissue fractionation of non-acidified teleost muscle tissue is 3.7 (C) and 5.6 (N). These limitations have to be considered although our values seem to support the commonly accepted range of fractionation since seagrass is indeed the predominant food item in the guts we analyzed. Still feeding experiments with our animals would be desirable to get more information on species-specific fractionation values.
We consider the combined evidence from gut content and stable isotope analysis sufficient to establish senescent seagrass leaves as important food sources for *N. acanthus* and *C. coutierei*. An additional uptake of the burrow lining, organic debris embedded within the sediment and small sediment grains is also considered very likely since these items are detached seagrass leaves anyway (embedded organic debris, burrow lining), and small sediment grains (as found in the burrow lining and as gained by the stirring behaviour) constituted a large part of the gut content of *N. acanthus* and *C. coutierei* and were also present in guts of *A. wardi*.
A. wardi is the first goby for which a seagrass diet could be established. In addition, seagrass epiphytes and crustaceans were present in its stomach. In another study on the gut contents of *A. wardi*, small crustaceans were also found but again the most dominant item was seagrass leaves (Liu et al. in prep.). *N. acanthus* and *C. coutierei* probably also take up epiphytes and meiofauna (which both have C:N ratios superior to seagrass), but these items are probably ground beyond recognition by their mandibles and gastric mills.
Gut contents were not analyzed for the cleaner shrimp and the amphipods but again their isotopic signature points a seagrass as a food source.
The only real exception is the bivalve which is strongly depleted in both 13C and 15N. Carlier (2007) likewise found that the bivalve *Loripes lacteus* was strongly depleted in both 13C and 15N. *L. lacteus* is known to harbour symbiotic bacteria which are considered responsible for the isotopic signature of their host. The high concentrations of dissolved nutrients resulting from the decay of seagrass material in burrows of *N. acanthus* could explain the presence of sulphate-reducing symbionts. We have not measured sulphur components but found high concentrations of Ammonium and reactive phosphate (Vonk et al. submitted). Felder (2001) reports that the bivalve *Phacoides pectinatus* which harbours sulphide-metabolising bacteria is associated with burrows of *Axianassa australis*, which are sulphide-rich.
The function of commensals found in *N. acanthus* burrows remains largely unknown. It could, however, be established that symbiotic organisms might not only be attracted by the shelter created but also by the food available.

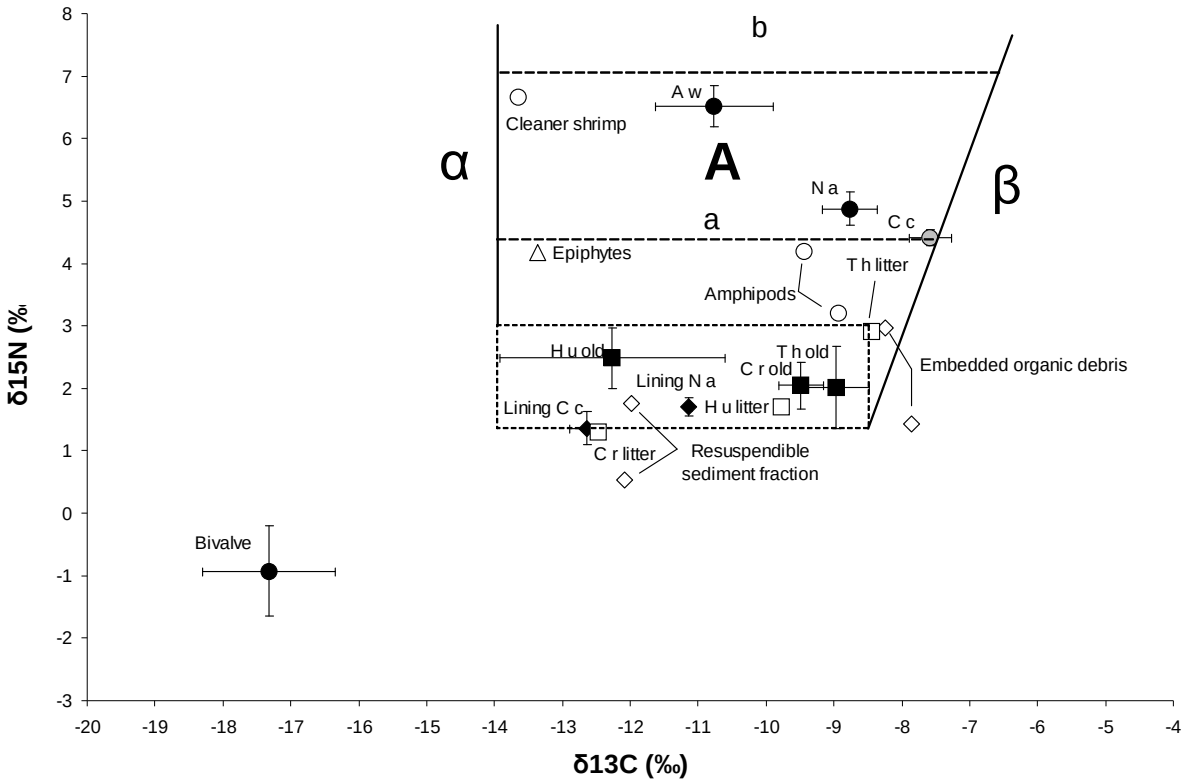


Fig. 1: carbon (x) and nitrogen (y) stable isotope signatures (±SD) of *Austrolethops wardi* (A w), *Neaxius acanthus* (N a), *Corallianassa coutierei* (C c), animals associated with *N. acanthus* and their potential food sources (*Thalassia hemprichii* (T h), *Cymodocea rotundata* (C r), *Halodule uninervis* (H u) and sediment).

References:

Carlier A, Riera P, Amouroux JM, Bodiou JY, Escoubeyrou K, Desmalades M, Caparros J, Grémare A (2007): A seasonal survey of the food web in the Lapalme Lagoon (northwestern Mediterranean) assessed by carbon and nitrogen stable isotope analysis, Estuarine, coastal and shelf science 73: 299-315

Felder, D.L. 2001. Diversity and ecological significance of deep-burrowing macrocrustaceans in coastal tropical waters of the Americas (Decapoda: Thalassinidea). *Interciencia* **26**, 440–449.

Gaston TF, Suthers IM (2004) Spatial variation in δ13C and δ15N of liver, muscle and bone in a rocky reef planktivorous fish: the relative contribution of sewage. J Exp Mar Biol Ecol 304:17–33

Macko SA, Lee WY, Parker PL (1982) Nitrogen and carbon isotope fractionation by two species of marine amphipods: laboratory and field studies. J Exp Mar Biol Ecol 63: 145–149

Parker PL, Anderson RK, Lawrence A (1989) A δ13C and δ15N tracer study of nutrition in aquaculture: *Penaeus vannamei* in a pond growout system. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Springer-Verlag, New York, p 288–303

Stephenson RL, Tan FC, Mann KH (1986) Use of stable carbon isotope ratios to compare plant material and potential consumers in a seagrass bed and a kelp bed in Nova Scotia, Canada. Mar Ecol Prog Ser 30:1–7

Vonk JA, Kneer D, Asmus H, Stapel J (2007) Shrimp burrows - an important pathway of litter decomposition in tropical seagrass meadows. Estuarine, Coastal and Shelf Science (accepted.)

Yokoyama H, Tamaki A, Harada K, Shimoda K, Koyama K, Ishisi Y (2005): Variability of diet-tissue isotopic fractionation in estuarine macrozoobenthos, Marine Ecology Progress Series 296: 115-128

3. The feeding habits of *Austrolethops wardi*, a gobiid fish inhabiting burrows of the thalassinid shrimp *Neaxius acanthus*
(prepared for Estuarine Coastal and Shelf Sciences)

The feeding habit of *Austrolethops wardi* (Gobiidae) in the seagrass beds of Barrang Lompo and Bone Batang island in the Spermonde Archipelago was investigated through gut content analysis. The feeding preferences of this species on both islands are very similar but uncommon for this speciose family. In this study *A. wardi,* a permanent inhabitant of burrows of *Neaxius acanthus*, fed almost exclusively on seagrass (100 % frequency of occurrence). However, animal food (< 1 %) occurred in the guts as well, predominantly in term of copepods and in lesser degree other small invertebrates. These results indicate that animal food is of little importance for *A. wardi* and might be accidentally ingested while feeding on seagrass parts. Some specimens even contained no parts of animal food. Stable isotope analysis was additionally performed and supports the assumption that this herbivorous species rely upon on seagrass collected by *N. acanthus.* Seagrass as food source has not been described in previous food habit studies for Gobiidae.

3. Ergebnisse aus den Expeditionen in den Spermonde Archipel

Ziel dieser Untersuchungen war die quantitative Charakterisierung der tropischen Seegraswiesen und ihrer Sukzessionstufen. Dabei sollten folgende Fragestellungen beantwortet werden:

a)Wie verändern sich die biotischen und abiotischen Faktoren in Seegraswiesen entlang von 2 Transekten vom Riffrand zum Strand (Artenzusammensetzung, Biomasse, Produktion), die in NO und SW-Richtung (in Windrichtung und geschützt zum Monsun) verlaufen

b) Worin liegen die Unterschiede beim Vergleich von Inseln in Küstennähe, in der Mitte des Schelfs und am Schelfrand hinsichtlich Artenzusammensetzung, Biomasse und der Primär-, Sekundär- und Tertiärproduktion von Seegraswiesen.

c) Welche Typen von Seegraswiesen treten auf, und durch welche Komponenten lassen sie sich charakterisieren.

Ergebnisse der Expedition im Mai 2004:
Um anthropogene Einflüsse auf die Seegraswiesen des Spermonde-Archipels zu untersuchen wurden zunächst, die qualitative und quantitative Zusammensetzung der Seegraswiesen an hand von Transekten erfasst, die von der Strandzone über die eigentliche Seegraswiese bis hin zum Riffsaum verlaufen.
Im Mai 2004 wurden die Seegraswiesen von 22 Inseln des Spermondearchipels qualitativ untersucht (Abbildung 1).

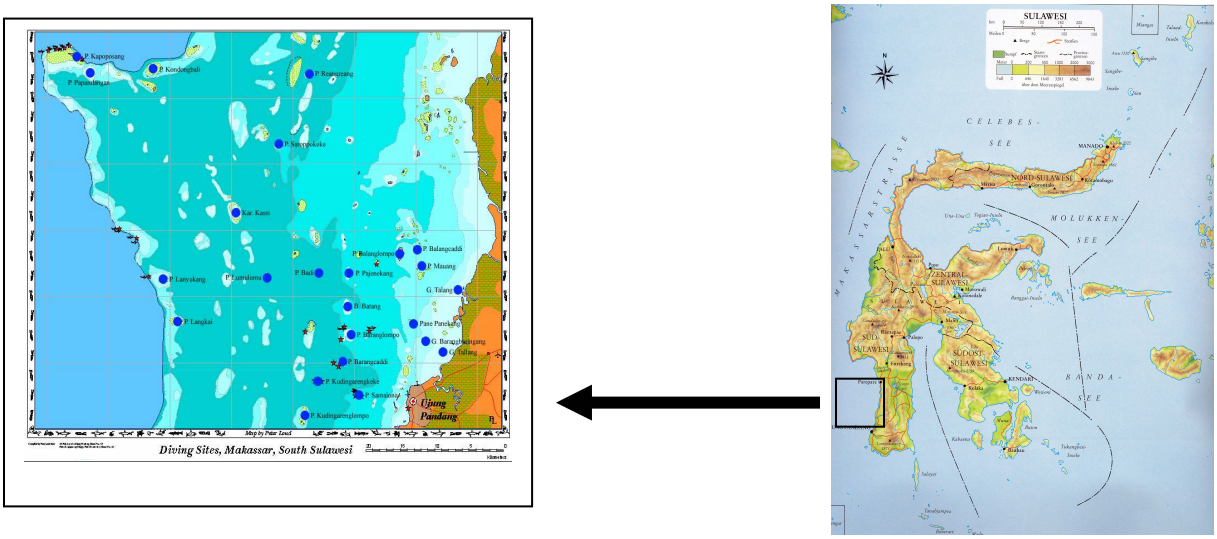


Abb. 1) Untersuchungsgebiet des Spermondearchipels im Südwesten der Insel Sulawesi. Im Bereich der blau markierten Inseln wurde die Seegrasgemeinschaft qualitativ untersucht.

Tab. 1: Nachgewiesene Seegrasarten im Spermonde-Archipel (Mai,September2004;März 2005) und bei Puntando (SW- Sulawesi, Indonesien) (Arten des Gezeitenbereichs wurden fett gedruckt dargestellt).

	Spermonde	Puntondo
Familie *Hydrocharitaceae*		
Enhalus acoroides (L.f.) Royle	+	+
Thalassia hemprichii (Ehrenberg)Ascherson	+	+
Halophila minor (Zollinger) den Hartog	+	
Halophila ovalis R.Brown	+	+
Halophila ovata Gaudichaud	+	
+		
Halophila spinulosa(R.Brown)Ascherson		+
Familie *Cymodoceaceae*		
***Halodule pinifolia* (Miki) den Hartog**	+	+
***Halodule uninervis* (Forskål) Ascherson**	+	+
Cymodocea rotundata Ehrenberg &Hemprich exAscherson	+	+
Cymodocea serrulata (R.Brown)Ascherson & Magnus	+	+
Syringodium isoetifolium (Ascherson) Dandy	+	+
Thalassodendron ciliatum(Forskål) denHartog		(Bira)

Die Artenzusammensetzung der Seegraswiesen zeigte eine unterschiedliche Kombination je nach den herrschenden Bedingungen. Pionierstadien bestehend aus nur einer Art (*Halophila ovalis*) standen reiche Beständen fortgeschrittener Sukzessionsstadien gegenüber (*Enhalus acoroides; Cymodocea rotundata, Thallassia hemprichii*). Bei Anwesenheit von Bioturbatoren gingen niedrige Seegrasarten zurück, während solche mit kräftigen Wurzelstock, wie *Enhalus acoroides* diesem Stressfaktor standhielten.
Im September 2004 wurde gezielt die Makrofauna in Seegraswiesen an 5 Inseln quantitativ erfasst.

Ergebnisse der Expeditionen im März und September 2005

Im März 2005 wurden je 2 Transekte einer küstennahen Insel (Barranglompo), einer Insel im zentralen Bereich des Archipels (Sarappokeke) und einer Insel am äußeren Schelfrand (Kapopposang) in Bezug auf die Seegrasvegetation (Bedeckungsgrad, Sprossdichte, Biomasse), die Epiphyten, die größere Makrofauna und die kleinere Endofauna quantitativ beprobt. Um Aussagen über die Fischfauna dieser Lebensgemeinschaft zu erhalten wurden auf je 3, 100m langen Transekten, die senkrecht zum Haupttransekt verliefen, *Visual census* Zählungen der vorhandenen Fischarten durchgeführt, die sofort protokolliert wurden und zur Kontrolle auf Video festgehalten wurde. Die Transekte zum Visual census wurden zu Beginn (strandnaher Bereich), in der Mitte (Zentrum der Seegraswiese) und am Ende (Übergangsbereich zum Korallenriff) des Hauptransektes durchgeführt. Diese räumliche Anordnung gewährleistete die gleiche Tiefenlinie pro Transekt. Die Ergebnisse aus den quantitativen Auswertungen sind noch in Bearbeitung.

Diese Untersuchungen mit Unterstützung eines Studententeams der UNHAS durchgeführt. Die Beprobungen der Transekte werden im Juni selbständig von dem Indonesischen Team wiederholt und dann im September 2005 erneut beprobt.

Die Besiedlung mit Makrofauna zeigt deutliche Unterschiede zu Seegraswiesen der gemäßigten Zonen, während in den gemäßigten Bereichen des Atlantik das Maximum der Biomasse an assoziierter Fauna bei der kleineren Makrofauna insbesondere den Weidegängern (benthic grazer) liegt, ist diese Gruppe in den untersuchten tropischen Wiesen des Spermonde Archipels weitgehend unterrepräsentiert. Hier dominieren große Filtrierer (Schwämme und Bivalvier der Gattung *Pinna*) und größere epibenthische Weidegänger (Seeigel der Gattungen *Tripneustes* und *Mespilia*).
Auffällig war die Artenarmut der Makrofauna in den üppig entwickelten Seegraswiesen vor der Insel Kapopposang

Die trophische Gruppe der Weidegänger wird in dem untersuchten Bereich vor allem in Küstennähe von großen Jungfischschwärmen der Gattung *Siganus* übernommen, die vor allem den Epiphytenbewuchs des großen Seegrases *Enhalus acoroides* abweiden und dadurch vermutlich auch zu dessen Wuchsleistung beitragen. Diese Fischgattung ist auch von kommerziellem Interesse bei der lokalen Inselfischerei.

Weitere Untersuchungen konzentrierten sich auf küstennahe Seegraswiesen am Südrand des Untersuchungsgebietes in der Bucht von Puntondo. Hier kommen neben Seegraswiesen auf koralligenem Sediment (wie auf den untersuchten Inseln im Spermondearchipel) auch solche auf terrigenem Sediment vor. An diesem Standort unmittelbar an der Küste wurden im April 2005, 2 zusätzliche Transekte quantitativ untersucht.

Verschiedene Fischassoziationen kamen in verschiedenen Seegraswiesentypen vor. Ausschlaggebend war dabei die Seegrasdichte, die Wassertiefe und die Nähe zu benachbarten Korallenriffbiotopen. Beach Seine- Fänge ermöglichten die genaue taxonomische Einordnung der Fänge und eine Untersuchung der Grössenspektren. Während in flachen Bereichen der Seegraswiesen offenbar mehr Jungfische vorkamen, überwogen in den tieferen Bereichen größere Individuen, aber in geringerer Anzahl.

Direkte Einflüsse durch den Menschen sind in den Seegraswiesen des Spermonde-Archipels auf kleiner bis mittlerer räumlicher Skala kaum spürbar, obwohl Überfischung des gesamten Schelfareals die Struktur des Nahrungsnetzes aller Schelfökosysteme stark verändert hat. Leider existieren bisher nicht einmal kleinräumige ungestörte Areale, in denen die Fischerei untersagt ist.

Ergebnisse der Expeditionen 2006:

Im Januar/Februar 2006 wurden die Seegrasbiomassen, Makrofaunabiomassen und die Untersuchungen der Fischbestände weiter analysiert .

Bestimmungen der Seegrasbiomasse erbrachten auffällige Unterschiede in der Seegrasdichte und dem jeweiligen Standort. Während im zentralen Schelfbereich, die Seegrasbiomasse nur schwach entwickelt war und durch kleine Seegrasarten (*Cymodocea* und *Halodule*-arten) aufgebaut wurde, bildeten sowohl im Küstenbereich der Hauptinsel Sulawesi als auch am äußeren Schelfrand hohe Biomassen und große Seegrasarten (*Enhalus acoroides* und *Thallassia hemprichii*) die Gemeinschaft. Durch den Vergleich der Seegrasbiomassen mit den Nährstoffdaten (Teilprojekt Schiel), konnte dies einerseits mit dem monsunbedingten Auftrieb nährstoffreichen Tiefenwassers an der Westseite des Schelfs, andererseits mit der Nährstofffracht der Flüsse Sulawesis im küstennahen Bereich des Schelfs in Zusammenhang gebracht werden. Die Seegraswiesen des zentralen Schelfbereichs stellen dagegen deutlich oligotrophe System dar.

Um den Einfluss des Auftriebsgeschehens auf die Primärproduktion des Seegrases zu bestimmen, wurden im März 2006 Primärproduktionsmessungen nach der Bell jar Methode in der Seegraswiese bei Kapoposang (äußerer Schelf) durchgeführt. Die Auswertung dieser Versuche ist noch in Bearbeitung. Erste Ergebnisse ergaben jedoch extrem hohe Sauerstoffgehalte (Sättigung bis zu 160%) während des Tages und extrem niedrige Werte während der Nacht (bis 50% Sättigung). Durch die vergleichsweise hohen Nährstoffgehalte verbunden mit dem hohen Lichtangebot wird die Produktion in den Seegraswiesen derart angeheizt, das starke Sauerstofffluktuationen die Folge sind.

Ergebnisse der Besiedlung mit Makrofauna zeigen deutliche Unterschiede zu Seegraswiesen der gemäßigten Zonen, während in den gemäßigten Bereichen des Atlantik das Maximum der Biomasse an assoziierter Fauna bei der kleineren Makrofauna insbesondere den Weidegängern (benthic grazer) liegt, ist diese Gruppe in den untersuchten tropischen Wiesen des Spermonde Archipels weitgehend unterrepräsentiert. Hier dominieren große Filtrierer (Schwämme und Bivalvier der Gattung *Pinna*) und größere epibenthische Weidegänger (Seeigel der Gattungen *Tripneustes* und *Mespilia*).
Auffällig war die Artenarmut der Makrofauna in den üppig entwickelten Seegraswiesen vor der Insel Kapoposang. Hier vermuten wir einen Zusammenhang mit den hohen Sauerstofffluktuationen im Wasser hervorgerufen von der, durch das lokal hohe Nährstoffangebot (Auftrieb) angeheizten Primärproduktion der Seegräser.

Die trophische Gruppe der Weidegänger wird in dem untersuchten Bereich vor allem in Küstennähe von großen Jungfischschwärmen der Gattung *Siganus* übernommen, die vor allem den Epiphytenbewuchs des großen Seegrases *Enhalus acoroides* abweiden und dadurch vermutlich auch zu dessen Wuchsleistung beitragen. Diese Fischgattung ist auch von kommerziellem Interesse bei der lokalen Inselfischerei. Im März 2006 wurde ein Ausschluss/Einschlussexperiment mit diesen Fischen durchgeführt, um den Zusammenhang zwischen Grazing –Intensität und Epiphytendichte zu quantifizieren. Doch leider war durch die verlängerte Regenzeit offenbar die Laichzeit der Fische verzögert, da zu Versuchsbeginn die 0-Gruppen der *Siganus* –Arten deutlich kleiner waren als im Vorjahr zur gleichen Zeit, und die Individuen daher durch die Maschen der vorbereiteten Käfige schlüpften. Das Experiment im Jahr 2007 im Rahmen einer Diplomarbeit wiederholt.
Weitere Untersuchungen konzentrierten sich auf küstennahe Seegraswiesen in der Bucht von Puntondo.

Ergänzende Unterlagen zum TP 3.2.:

1. Kurz- Zusammenfassung

Seegraswiesenökosysteme sind wichtige Bestandteile tropischer Riffe, die vor allem als Aufwuchsgebiet für junge Rifffische eine große Rolle spielen und durch vielfältige Interaktionen mit den Korallenriffen eng verbunden sind.

Die Küstengebiete Indonesiens unterliegen einer Vielzahl von Nutzungen wie Fischerei, Aquakultur und Tourismus. Gleichzeitig wirkt sich das mit der raschen Entwicklung des Landes verbundene Wachstum der Küstenstädte sowie die fortschreitende Intensivierung der Land - und Forstwirtschaft auf die marinen Küstenökosysteme aus. Umweltverschmutzung und Sedimenteintrag nach Erosion durch Entwaldung schaffen insbesondere für Korallenriffe und Seegraswiesen große Probleme. Die Pläne in bestimmten Gebieten (z.B. Spermonde- Archipelago) den Ökotourismus zu etablieren, erfordern viele spezielle, harmonisierende Maßnahmen, um ein Nebeneinander dieser Nutzung mit der durch Fischerei und Aquakultur zu ermöglichen und die Funktionsfähigkeit dieser Ökosysteme langfristig nachhaltig zu bewahren.

Die Rolle der Beeinflussung der tropischen Seegraswiesen und ihrer dominanten Glieder durch den Menschen (Algenfarmen und Fischerei) wird erforscht. Das Nahrungsnetz und der Wirkungsgrad des trophischen Transfers und der trophischen Gruppen wird im Hinblick auf die Biodiversität dieser Systeme und die Störeinflüsse durch den Menschen untersucht.

2. Vergleich des Standes des Vorhabens mit der ursprünglichen Arbeits- Zeit- und Aufgabenplanung.

Es konnten alle ursprünglich für das Teilprojekt vorgesehenen, geplanten Expeditionen und Schiffsausfahrten durchgeführt werden.
Durch die verlängerte Regenzeit konnte der geplante Versuch zum Einfluss herbivorer Fische auf das Wachstum der Seegräser leider nicht durchgeführt werden, da die fische während der Messkampagne die erforderliche Größe noch nicht erreicht hatten. Diese Versuche wurden daher im Rahmen einer Diplomarbeit im Jahr 2007 nachgeholt. Die Ergebnisse dazu liegen zu Beginn des Jahres 2008 vor.

Die im Rahmen dieses Teilprojektes verfassten Dissertationen, Diplomarbeiten und Publikationen sind wie folgt aufgelistet.

Doktorarbeiten:

Blankenhorn S.U. (2007) Influence of algal cultures and local fishery on seagrass beds in South Sulawesi, Indonesia and its sustainable use. PhD-Thesis, Alfred- Wegener-Institute für Polar- und Meeresforschung and Universität Bremen

Diplomarbeiten:

Kneer D (2006). The role of *Neaxius acanthus* (Thalassinidea: Strahlaxiidae) and its burrows in a tropical seagrass meadow, with some remarks on *Corallianassa coutierei* (Thalassinidea: Callianassidae). Diplomarbeit, Freie Universität Berlin.

Liu H.T.H (2007). Biodiversity and trophic position of the family Gobiidae (Pisces) in an Indonesian seagrass bed around Barrang Lompo, Spermonde Archipelago, Indonesia, Diplomarbeit, Universität Wien (Abschluß Frühjahr 2008)

Schauerte P (2005). The effect of synaptid holothurians (Synaptidae) on epiphyte biomass and macrophyte growth of the tropical seagrass *Enhalus acoroides* in Indonesian seagrass beds. Diplomarbeit, Universität Köln

Publikationen, die aus TP 3.2 in SPICE 1 hervorgegangen sind:

Blankenhorn SU, Asmus H (2007) Effects of seaweed farming on seagrass shoot density and biomass. Marine Ecology Progress Series (submitted)

Blankenhorn SU, Asmus H (2007) Fishermen's perception of artisanal fisheries and implications for management in a coastal bay in South Sulawesi, Indonesia. Ocean and Marine Management (submitted)

Blankenhorn SU, Asmus H (2007) Variations in fish stocks and artisanal gill net fishery in an Indonesian seagrass bed . Fisherments management and Ecology (submitted).

Blankenhorn SU, Asmus H (2007) Seaweed farming in traditional fishing villages: An example from South Sulawesi, Indonesia. Ocean and Marine Management (submitted)

Kneer D, Asmus H, Ahnelt H, Vonk JA (2007) Records of *Austrolethops wardi* (Teleostei: Gobiidae) as an inhabitant of burrows of the thalassinid shrimp *Neaxius acanthus* in tropical seagrass beds of the Spermonde Archipelago, Sulawesi, Indonesia. Journal of Fish Biology (in press).

Kneer D., Asmus H., Vonk J. A (2008) Evidence for seagrass as food source for *Neaxius acanthus* (Thalassinidea: Strahlaxiidae) and the commensal community in its burrows,with remarks on the behaviour of *Neaxius acanthus* and the diet and burrow morphology of *Corallianassa coutierei* (Thalassinidea: Callianassidae) Ecology (in prep.)

Vonk JA, Kneer D, Asmus H, Stapel J (2007) Shrimp burrows - an important pathway of litter decomposition in tropical seagrass meadows. Estuarine, Coastal and Shelf Science (accepted.)

Liu HTH, Kneer D, Asmus H, Ahnelt, H (2008) The feeding habits of *Austrolethops wardi*, a gobiid fish inhabiting burrows of the thalassinid shrimp *Neaxius acanthus.* Estuarine, Coastal and shelf Science (in prep.)

3. Änderungen der Aussichten für die Erreichung der Ziele des Vorhabens innerhalb des angegebenen Zeitraumes.

Die Aussichten zur Ereichung der Ziele des Vorhabens haben sich während des angegebenen Zeitraumes nicht geändert.

4. Sind von dritter Seite Ergebnisse bekannt geworden, die für die Durchführung der Ziele relevant sind?

Der Wissensgewinn über tropische Seegraswiesen erfolgt weltweit nur zögerlich, obwohl diese Lebensgemeinschaften wegen ihrer Ausdehnung eine größere Bedeutung zukommen sollte als der Forschungsaktivität bisher entspricht. Die Brennpunkte der Forschung liegen dabei vor der australischen Küste und in der Karibik. Neue Ergebnisse aus anderen Bereichen haben für die Zielsetzung bestenfalls nur ergänzenden Charakter.
So wurde im Spermonde- Archipel eine neue Seegrasart *Halophila sulawesii* beschrieben, die vermutlich die tiefbilden Seegraswiese jenseits der Riffe dominiert und zwischen 10 und 50m Wassertiefe zu finden ist. Die Untersuchungen zu dieser neuen Art sind rein taxonomisch –morphologischer Natur und haben bisher noch keine ökologischen Anwendungen gefunden.

5. Sind Änderungen in der Zielsetzung notwendig ?
Es sind bisher keine Änderungen der Zielsetzung notwendig

6. Fortschreibung des Verwertungsplans

-Wirtschaftliche Erfolgsausichten
Es wurden keine direkten wirtschaftlichen Ziele formuliert und es werden daher auch keine direkten wirtschaftlichen Erfolge erwartet.

-Wissenschaftliche und/oder technische Erfolgsaussichten
Die wissenschaftlichen und technischen Erfolgsaussichten sind aufgrund der Vorarbeiten im Rahmen von SPICE 2004-2007 sehr gut. Dissertationen, Diplomarbeiten und die Ergebnisse aus den Messkampagnen sind bereits publiziert oder werden direkt in wissenschaftlichen Publikationen international begutachteter Zeitschriften einfließen.

Die geplanten Arbeiten in Seegraswiesen sind für die Bewertung der marinen Umwelt unerlässlich. Sie stellen die wissenschaftliche Grundlage für die Entwicklung von Managementempfehlungen für die nachhaltige Ressourcen-Nutzung dar.
Es ist daher geplant, die Ergebnisse aus dem geplanten Projekt über den Indonesischen Partner der Öffentlichkeit in Indonesien zur Verfügung zu stellen und über die globalen Rahmenprogramme COREMAP und LOICZ in nationale und Internationale Netzwerke einzubinden. Eine enge Zusammenarbeit mit der Hasanuddin Universität Makassar, dem ZMT und der Universität Bremen ist für das Teilvorhaben unabdingbar.

Wissenschaftliche und wirtschaftliche Anschlussfähigkeit
Die Arbeiten der Seegrasgruppe werden erfolgreich auch in der kommenden Phase des Vorhabens fortgesetzt. Dies wird in Form von wissenschaftlichen Publikationen, Öffentlichkeitsarbeit und Entwicklung weiterer Forschungsanträge durchgeführt werden.
Darüber hinaus hat sich im Bereich Seegrasforschung an der Hassanuddin Universität eine kleine Expertengruppe etabliert, die über die Projektzeit hinaus einen lebendigen Wissenschaftsaustausch betreiben wird zwischen dem Gastland und deutschen Forschergruppen (Gemeinsame Masterthesis von Dody Priosambodo, Hassanuddin University – AWI Sylt).