UNIVERSIDADE FEDERAL DE PERNAMBUCO
CENTRO DE TECNOLOGIA E GEOCIÊNCIAS
DEPARTAMENTO DE OCEANOGRAFIA

Biologia populacional de *Pomacanthus paru* (Teleostei: Pomacanthidae) e análise da sustentabilidade de captura do *bycatch* de peixes ornamentais.


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Tese submetida ao curso de Doutorado do Programa de Pós Graduação em Oceanografia da Universidade Federal de Pernambuco, como requisito para obtenção do título de Doutor em Oceanografia.

Orientadora: Drª Maria Elisabeth de Araújo
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Sumário

Resumo

Pomacanthus paru tem grande importância para o aquarismo brasileiro, além de ser a segunda espécie marinha mais exportada desde 2000. Os objetivos desta pesquisa foram os seguintes: (a) descrever a biologia reprodutiva e determinar a idade e o crescimento de *P. paru*, (b) analisar a diversidade genética de *P. paru* e *P. arcuatus* a partir do DNA mitocondrial, (c) registrar as espécies de peixes ornamentais capturadas como *bycatch* através da pesca com armadilhas e propor uma metodologia para determinar a sustentabilidade de captura das principais espécies ornamentais marinhas no Brasil. Amostragens semanais foram realizadas durante o período de março 2006 a fevereiro 2007. Os exemplares menores de 15 cm foram comprados de coletores de peixes ornamentais. Os peixes foram medidos, pesados, e as gônadas e os otólitos foram removidos. Amostras de músculo e do fígado foram armazenadas para o estudo genético. A região 16S rDNA foi amplificada e sequências do ADN mitocondrial foram utilizadas para a análise dos haplótipos. Para a proposição da metodologia de sustentabilidade de captura, cada espécie foi avaliada segundo dois grupos de critérios: (1) vulnerabilidade relativa à captura pelas armadilhas; (2) resiliência da população relacionada à mortalidade devido à pressão pesqueira. No total, 236 indivíduos foram analisados, sendo 139 fêmeas, 86 machos e 11 que não tiveram o sexo definido. *P. paru* é uma espécie gonocorística, que se reproduz ao longo do ano, com as fêmeas apresentando dois picos de desova (Fevereiro-Março/Setembro-Outubro). As fêmeas foram mais abundantes em todos os meses, exceto em outubro. O L_{50} é atingido aos 30 cm para as fêmeas e 35 cm para os machos. A idade dos indivíduos amostrados de *P. paru* variou de 1 a 27 anos. *P. paru* atinge o comprimento máximo lentamente e tem um longo ciclo de vida. Para *P. paru* seis haplótipos foram encontrados, enquanto em *P. arcuatus* apenas um, incluindo os espécimes do Caribe. Foram registradas como *bycatch*, 19 espécies pertencentes a 10 famílias. As espécies cujas capturas foram menos sustentáveis foram *Holacanthus tricolor*, *H. ciliaris*, *Chaetodon striatus* e *P. paru.* O manejo de *P. paru* deve incluir medidas baseadas no tamanho de primeira maturação sexual, bem como nos parâmetros de crescimento aqui estabelecidos. Os resultados relacionados à genética representam um primeiro passo para se compreender as estruturas populacionais de espécies tão próximas filogenética e ecologicamente como *P. paru* e *P. arcuatus.* A nova metodologia aqui proposta deverá ser uma ferramenta útil e alternativa para o manejo e avaliação da sustentabilidade de captura das espécies oriundas de pescarias multi-específicas, com poucos dados disponíveis e que ocorrem em áreas de alta riqueza, como os recifes.

Palavras-chave: *Pomacanthus paru*, reprodução, idade, crescimento, *bycatch*

Abstract

Pomacanthus paru presents great importance in the Brazilian aquarium trade, being the second most exported species since 2000. The aims of this research were (a) to describe the reproductive biology and to determine the age and growth, (b) to analyze the genetic diversity of *P. paru* and *P. arcuatus* from mt-DNA, (c) to record ornamental fish *bycatch* and to propose a methodology to assess the catch sustainability of ornamental fish *bycatch* from the major Brazilian families in the aquarium trade. Weekly samplings were performed from March 2006 to February 2007. Individuals smaller than 15 cm were bought from collectors of ornamental fish. The fish were measured, weighed and the gonads and otoliths were removed. Samples of tissue from muscle and liver were stored for the genetic study. A region of the 16S rDNA was amplified and sequences of the mtDNA were used for haplotypes analysis. To assess the catch sustainability of ornamental fish *bycatch*, each species was assessed based on two groups of criteria: (1) relative vulnerability to capture; and (2) population resilience regarding mortality due to fishing efforts. Of the 236 individuals sexed, 139 were female, 86 were male and 11 were indefinite. *P. paru* is a gonochoric species, which is reproductive activite all year-round, with females presenting two spawning peaks (February-March/September-October). Females were more abundant at all months, except October. The L_{50} is reached at 30 cm for females and 35 cm for males. The ages of the fish in the sample varied from 1 to 27 years. *P. paru* attains its maximal size slowly and has a long lifespan. For *P. paru* six haplotypes were recorded, while only one was registered for *P. arcuatus*, including Caribbean species. Nineteen species from 10 families were recorded. The species least likely to have sustainable catch rates were *Holacanthus tricolor*, *H. ciliaris*, *Chaetodon striatus* and *P. paru.* The management of this latter species should include measures based on its size at maturity as well as in growth parameters established in this research. The considerations regarding genetic represent a first step toward understanding the population structures of species as phylogenetically and ecologically close as *Pomacanthus paru* and *P. arcuatus.* The proposed methodology is a useful alternative for management efforts and the assessment of catch sustainability in newly-developed multi-species fisheries with limited available data, which occur in areas of high species richness such as reefs.

Key-words: *Pomacanthus paru*, reproduction, age, growth, *bycatch*

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Introdução

Os peixes da família Pomacanthidae, conhecidos como peixes-anjo, possuem o corpo alto e comprimido lateralmente, algumas vezes quase circular (Cervigón, 1993). São bastante parecidos com os peixes borboleta da família Chaetodontidae (Smith & Heemstra, 1991) e Ephippidae (Menezes & Figueiredo, 1985). Apresentam boca pequena, terminal e protátil, extremidade inferior do pré-opérculo com um forte e largo espinho e escamas ctenóides pequenas (Menezes & Figueiredo, 1985; Cervigón, 1993). São de tamanho médio e possuem padrões de colorido variados e contrastantes, diferindo notadamente entre os jovens e adultos (Nelson, 1994). É uma das famílias mais procuradas pelos aquaristas, sendo o colorido a principal razão desta popularidade (Allen et al., 1998).

Os pomacantídeos distribuem-se por todas as latitudes tropicais e subtropicais. Estão geralmente associados às águas costeiras com desenvolvimento de recifes ou pedras (Cervigón, 1993; Allen, 1979) em profundidades inferiores a 20m e raramente abaixo dos 50m (Nelson, 2006). A família Pomacanthidae possui 82 espécies e oito gêneros (Nelson, 2006) com mais da metade das espécies sendo pertencentes aos gêneros *Centropyge* e *Pomacanthus* (Nelson, 1994). Destas, 89% do total ocorrem nos oceanos Índico e Pacífico. No Atlântico, a maioria das espécies ocorre na porção oeste deste oceano, comumente referidas para o mar do Caribe. Apenas uma espécie é exclusiva do oeste da África e outra do Atlântico Central (Ascenção). Para Austrália são registradas 24 espécies, sendo considerado o país com a maior diversidade de pomacantídeos (Allen et al., 1998). O gênero *Pomacanthus* está representado por 13 espécies, além de 4 híbridos (Allen et al., 1998). Estas espécies podem ser encontradas em águas rasas em regiões de recifes e pedras, em grupos ou isoladamente (Menezes & Figueiredo, 1985).

Estudos sobre a ecologia dos pomacantídeos são numerosos (e.g. Randall & Hartman, 1968; Hourigan et al., 1989; Roberts et al., 1992; Sakai & Kohda, 1997; Kuwamura, 1998;

Kulbick & Sarramégna, 1999; Sazima et al., 1999; Aburto-Oropeza et al., 2000; Hamagushi et al., 2002; Bellwood et al., 2004). Entretanto, informações sobre seus aspectos populacionais como idade, crescimento e longevidade são relativamente escassas. A única espécie da família avaliada quanto à idade e o crescimento foi *Pomacanthus imperator*, onde as escamas foram utilizadas para determinação destes parâmetros (Chung & Woo, 1999). Os pomacantídeos foram geneticamente estudados principalmente no Atlântico Sul. Foram realizadas análises com marcadores citogenéticos (Galetti et al., 2006), enzimáticos (Chung & Woo, 1998), e mitocondriais (Bellwood et al., 2004; Bowen et al., 2006), principalmente com o objetivo de responder questões taxonômicas e evolutivas. Uma vez que os pomacantídeos encontram-se sob forte pressão pesqueira, informações genéticas continuam sendo necessárias a fim de obter uma medida acurada da biodiversidade (Affonso & Galetti, 2007).

Dentre as espécies de pomacantídeos, aquelas do gênero *Centropyge* são as mais estudadas principalmente no que se refere ao desenvolvimento gonadal e comportamento reprodutivo (Bauer & Bauer, 1981; Moyer et al*.*, 1983; Aldenhoven, 1986; Sakai, 1986; Sakai & Kohda, 1997). Alguns estudos foram realizados enfocando aspectos reprodutivos para os gêneros *Holacanthus* e *Pomacanthus*, tais como tamanho de primeira maturação sexual, fecundidade (n = 2) (Aiken, 1983) e época de desova (n = 21) (Munro et al., 1973). Porém, o tamanho amostral de *P. paru* foi considerado pequeno pelos autores para realizar comparações com outras pesquisas.

Os principais componentes da dieta destes indivíduos são as esponjas e algas (Randall, 1967; Randall & Hartman, 1968). Quando jovens, complementam sua dieta retirando ectoparasitas de peixes de maior porte (Allen et al., 1998; Sazima et al., 1999). São espécies que normalmente formam pares, entretanto pequenos haréns foram registrados (Allen et al., 1998).

A espécie *P. paru* (Bloch, 1787) distribui-se desde a Flórida até o sudeste do Brasil. É relativamente comum no litoral brasileiro (Menezes & Figueiredo, 1985), sendo mais abundante na região Sudeste quando comparada a Nordeste (Ferreira et al., 2004). Ocorre em profundidades entre 5 a 100 m (Allen, 1979). Atinge cerca de 40 cm de comprimento e quando adulto, possui o corpo enegrecido, tendo a cabeça uma coloração mais clara e as margens das escamas amareladas. Nos jovens o corpo é totalmente negro com cinco faixas amarelas verticais (Allen et al., 1998). O jovem de *P. paru* é muito parecido com a espécie *P. arcuatus*, diferindo no número de raios e principalmente na nadadeira caudal, que é truncada posteriormente em *P. arcuatus* e arredondada em *P. paru* (Menezes & Figueiredo, 1985).

O conhecimento do ciclo de vida de espécies como *P. paru*, que são extraídas diretamente do ambiente natural para o aquarismo, é fundamental para a sua conservação. Embora *P. paru* não se encontre na lista de espécies ameaçadas de extinção da IUCN (IUCN, 2008), ela é muito explorada no Brasil pela indústria aquariofilista (Monteiro-Neto et al., 2003). Devido à pressão pesqueira sobre os jovens, a abundância de *P. paru* em alguns pontos próximo a Guarapari (ES) está inversamente relacionada com a distância da costa, enquanto o número de adultos não difere (Gasparini et al., 2005). A abundância desta espécie é significativamente maior em áreas protegidas (Gasparini et al., 2005). Nos últimos anos, *P. paru* vem sendo listada como a segunda espécie mais exportada pelo Brasil (Monteiro-Neto et al., 2003; IBAMA, 2007). Entre o período de 1995 a 2000, somente o Estado do Ceará exportou 22.969 espécimes de *P. paru* (Monteiro-Neto et al., 2003) e, em 2007, 13.422 indivíduos foram exportados pelo Brasil (IBAMA, 2007). O ordenamento destas espécies vem sendo realizado através de uma instrução normativa do IBAMA (IN56/04), onde foram estabelecidas cotas específicas de exportação. Atualmente a cota de captura deste indivíduo é de 2.500 indivíduos/ano/empresa (IBAMA, 2004).

Grande parte das espécies marinhas comercializadas no aquarismo é de origem tropical e extraída dos recifes. O potencial para a sobrexploração desta atividade é alto, uma vez que indivíduos juvenis, na maioria das vezes, menores que 10 cm de comprimento e de muito valor são retirados em grandes quantidades (Wood, 2001). Além dos efeitos diretos causados pela extração de peixes para o aquarismo é consenso os danos provocados no ecossistema marinho pelas práticas destrutivas utilizadas para coletar estes organismos (Wood, 1985, 2001; Nottingham et al., 2005). Somada à pressão pesqueira sofrida por esta espécie por meio do aquarismo, *P. paru* é constantemente capturada como *bycatch* em armadilhas de pesca no Nordeste do Brasil (Feitosa et al., 2008). As armadilhas têm sido responsáveis pelo colapso de muitos recursos recifais (Wolff et al., 1999), principalmente devido à pouca seletividade do petrecho. No caso específico de *P. paru*, espécimes de 15 a 40 cm de comprimento foram pescadas pelas armadilhas (Feitosa et al., 2008). Dentre os peixes ornamentais capturados por este petrecho, aqueles dos gêneros *Acanthurus*, *Chaetodon*, *Bodianus*, *Holacanthus* e *Pomacanthus* são frequentemente os mais capturados (Garrisson et al., 1994; Wolff et al., 1999).

A sobrexploração de espécies de peixes marinhos pela captura para fins ornamentais e a destruição de habitats são apontados como os principais problemas gerados pelo aquarismo. Portanto, recomenda-se que sejam realizados levantamentos estatísticos sobre o número de peixes ornamentais envolvidos no mercado, além de estudos sobre a biologia e dinâmica populacional destas espécies (Andrews, 1990; Edwards & Shepherd, 1992; Wood, 2001).

A demanda global por peixes ornamentais tem resultado no aumento direto dos índices de coletas nos estoques naturais (Ogawa & Brown, 2001). Pouco se sabe sobre a dinâmica populacional destas espécies, pois a quantidade de espécies exportadas é baseada em entrevistas com coletores que muitas vezes não falam a realidade. A situação das populações de peixes recifais é inconclusiva, principalmente devido a variações sazonais naturais, bem como as alterações no esforço de pesca para um dado ano de estudo (Ogawa & Brown, 2001).

Nas últimas reuniões técnicas, promovidas pelo IBAMA, sobre a exploração de peixes ornamentais marinhos, foi enfatizada a carência de pesquisas sobre a biologia e dinâmica populacional das principais espécies exportadas pelo Brasil (e.g. *Holacanthus ciliaris* e *Pomacanthus paru*) (IBAMA, 2000, 2003). Desta forma, espera-se que os resultados obtidos na presente pesquisa forneçam subsídios fundamentais à criação de medidas de regulamentação, bem como proporcionem dados necessários ao adequado manejo de suas populações.

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Capítulo 1 - **Reprodução do Paru preto *Pomacanthus paru* (Teleostei: Pomacanthidae) no nordeste do Brasil.**

Chapter 1* - **Reproduction of the French Angelfish *Pomacanthus paru* (Teleostei: Pomacanthidae) from Northeastern Brazil.**

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* Capítulo em formato de artigo e padronizado de acordo com as normas da revista *ICES Journal of Marine Science.*

Reproduction of the French Angelfish *Pomacanthus paru* (Teleostei: Pomacanthidae) from Northeastern Brazil.

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Abstract

Pomacanthus paru is an important species for the Brazilian aquarium trade, being the second most exported. The aim of this study was to describe the reproductive biology of *P. paru.* Fish were caught as bycatch in trap fishery. Weekly samplings were performed from March 2006 to February 2007. Total length (TL), standard length (SL) and body weight (BW) were recorded. Gonadosomatic index (GSI) and batch fecundity were calculated. Individuals were sexed and the maturity stages were determined. The French angelfish is a gonochoristic fish and presents size dimorphism. Sex ratios were biased toward female at all months, except October. The L50 were different between sexes, being maturity reached at 30cm for females and 35 cm for males. *Pomacanthus paru* is reproductively active throughout the year, with females presenting two distinct spawning peaks. This species is mainly targeted by the aquarium trade, but it is also caught as bycatch. Despite the determination of the size at maturity, this information is not relevant for the aquarium trade management, as most commercialized ornamental fish are juveniles under 10 cm in standard length. It can be suggested that conservationist issues should be based on capture per area and the establishment of protected areas.

Key words: *Pomacanthus paru*, sex ratio, size at maturity, size dimorphism, spawning season.

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Introduction

Knowledge on fish reproduction is especially important for targeted species. Size at first maturity, reproductive behavior and fecundity are essential characteristics for the management of fisheries (Wootton, 1979; Jennings *et al*., 2001).

Pomacanthids, or angelfish, are among the most colorful and widely recognized group of reef fish (Thresher, 1982), and a favorite for divers and aquarists. Angelfish occur throughout tropical and subtropical seas and are associated with coral reefs. The biology of Pomacanthidae family has caused special concern among reef ichthyologists, particularly due to its habit of forming harems (Allen *et al*., 1998). The genera *Centropyge* and *Geniacanthus* (the smallest angelfish), are the most studied in terms of gonad development and reproductive behavior (Bauer and Bauer, 1981; Moyer *et al*., 1983; Aldenhoven, 1986; Sakai, 1986; Sakai and Kohda, 1997). The reproductive behavior of angelfish is relatively well known but there is little information on the spawning habits of *Pomacanthus* angelfish (Allen, 1979). *Pomacanthus* angelfish are known to be gonochoristic (Moyer *et al*., 1983; Thresher, 1984) but further studies are needed to state this for all members of the genus (Michael, 2004).

Pomacanthus paru is distributed from Florida, the Bahamas and northern Gulf of Mexico to Santa Catarina State - Brazil (Menezes *et al*., 2003). It is also reported for St. Paul's Rocks (Feitoza *et al*., 2003) and Ascension Island (Maugé, 1990) in the Central Atlantic and Gulf of Guinea. Maximal length is 41 cm (Cervigón, 1993). This species occurs in waters from 5 to 100 m, but juveniles and subadults are more common in shallow waters (Allen *et al*., 1998; Michael, 2004). The French angelfish is an omnivore. Sponges are its main food item, constituting nearly 75% of its diet (Randall, 1967). It appears that adult usually form stable pairs, although in areas where the fish is more common it is known to form small harems (Allen *et al*., 1998; Michael, 2004).

Most species collected for the Brazilian aquarium trade belong to the Pomacanthidae family (Araújo and Albuquerque Filho, 2005). *Pomacanthus paru* is an important species in this trade and has been one of the most exported species since 2000 (Araújo and Albuquerque Filho, 2005; Nottingham *et al.*, 2000; Monteiro–Neto *et al.*, 2003). During the period of 1995 and 2000, only the Ceará State (Northeastern Brazil) exported 22. 969 specimens of *P. paru* (Monteiro-Neto et al., 2003). This species is managed through a Federal Act (56/04) established in 2 004 with the purpose of managing activity through the establishment of export quotas. Besides the impact from aquarium trade on juveniles, this species is frequently caught by traps. Trap fisheries catch a broad length range of ornamental species (e.g. *Holacanthus ciliaris* and *Pomacanthus paru*), including individuals that make up the breeding stock (Feitosa *et al.*, 2008). There is no regulation for this type of fishery in Brazil.

Studies on reproduction are very important for fishery management (Fonteles – Filho, 1989), as management decisions are based on reproductive parameters, such as size at maturity. There have been few studies on the sexuality, sexual maturity, fecundity (Aiken, 1983) and spawning season of pomacanthids (Munro *et al*., 1973). For *P. paru* the sample sizes in the two studies cited were very small (four and 21 individuals, respectively). Thus, there is no substantial information on the population structure of this species.

The aim of the present study was to describe the reproductive biology of the *P. paru*. The specific goals of the study were to investigate (a) gonad development, (b) spawning season, (c) gonad morphology and histology, (d) fecundity and (e) size at sexual maturity in order to provide useful information for the management of the fishery targeting this species.

Materials and methods

Sample collection

The fish were collected from commercial fishing boats of the fishing colony of Itamaracá Island located on Pilar beach (7°45.30’S 34°49.44’W) (state of Pernambuco, Northeastern Brazil – Fig. 1). The specimens were caught as bycatch while trap fishing for the spotted goatfish (*Pseudupeneus maculatus*) and parrotfish (*Sparisoma* spp). In this fishery, traps are deployed unbaited next to reefs and hauled 6 hours later. Occasionally, depending on factors such as wind and currents, some traps are left for longer periods and only hauled after 48 hours.

Figure 1: Location of the sampled site.

Weekly samplings were carried out from March 2006 to February 2007. As individuals smaller than 15 cm are not caught by traps, they were bought from fish collectors between September 2006 and February 2007.

The fresh sampled fish were placed in polystyrene chests with ice and taken to the Laboratório de Nécton e Aquicultura of the Departamento de Oceanografia of Universidade Federal de Pernambuco. Total length (TL), standard length (SL) and body height (BH) and weight (BW) were recorded from the specimens to the nearest 1 mm and to the nearest 0.01 g. The gonadossomatic index (GSI) was used as an index of reproductive activity and was calculated as follows: [GSI = (GW/BW) x 100], in which GW is the gonadal weight (g) and BW is the fish weight. For the batch fecundity analysis, 0.100 g of the ovary were removed and separated in glycerine with the aid of a brush and knife; and the hydrated oocytes in this sample were counted (Hunter and Macewicz, 1985a; Hunter and Goldberg, 1980; Vazzoler, 1996). Relative fecundity was expressed as the number of eggs/gram of ovary-free body weight. Oocyte diameter was measured under a microscope at 100 x with a video camera attachment, using the ImageLab software program (Softium).

Sample processing

The gonads were dissected from the fish, weighed to the nearest 0.01 g, fixed in Bouin’s solution for 24 h and transferred to 70% ethanol for later sectioning. The mid portion of the gonad was embedded in paraffin, following standard histological techniques. The paraffin blocks were sectioned transversely at 6 μm thickness by a rotary microtome. Duplicate slides were prepared for each tissue and stained with Mayer’s hematoxylin-eosin, following standard histological procedures. Individuals were sexed and the maturity stages were determined adopting the stages proposed by Murphy and Taylor (1990), West (1990) and Yamaguchi *et al.* (2006) (Table 01). Sex and reproductive condition were determined for each individual based on histological evaluations under a microscope at 40 – 400 x magnification.

Table 1: Macroscopic and histological stages used to describe ovaries and testes of P*omacanthus paru* based in Murphy and Taylor (1990), West (1990) and Yamaguchi *et al.* (2006).

Gonad stage by sex	Main characteristics
Females	
Immature	Difficult to determine sex macroscopically. Ovaries small and threadlike, transparent. Most advanced oocytes are at peri-nucleolus stage or yolk vesicle stage.
Maturing	The gonads present reduced dimensions (≈ 6 cm in length and 1.80 cm in width). Ovaries varies in color from pink to pale yellow. Most oocytes are in early vitellogenesis stage. Oocytes in late vitellogenesis stage also can be seen. Besides, brown bodies are also observed.
Ripening	Ovaries are yellow in color. An increase in blood vessels, volume and size of the structure are recorded. Most oocytes are in late vitellogenesis stage. Few oocytes are in early vitellogenesis stage. Brown bodies are also registered.
Spawning	Gonads very developed. Ovaries coloration varies from yellow to orange. Migratory nucleus, hydrating or hydrated oocytes. Brown bodies recorded.
Resting	Most advanced oocytes are at peri-nucleolus stage or yolk vesicle stage. Due to the diameter of the gonad and the thickness of the gonad wall, it was possible to differ the resting ovaries differ from the immature ones.

Gonad stage by sex	Main characteristics
Males	
Immature	Difficult to determine sex macroscopically. Testes small and threadlike, transparent. Testes with spermatogonia in the first spermatogenesis stage.
Maturing	Testes transparent or pale white. Tissue predominantly comprised of primary and secondary spematocytes. Few quantities of spermatids in lobules.
Ripening	White testes. Tissue consists predominantly of spermatocytes, spermatids and spermatozoa. Spermatozoa present in lobules, but none in spermatic ducts.
Spawning	White testes, enlarged. Mature spermatozoa fill the spermatic ducts.
Spent	Testes dull brown in colour. Developed lobules containing few remaining sperm.
Resting	Flat, white-grayish testes. Spermatogonia in the first spermatogenesis stage.

Statistical analysis

The Mann-Whitney test was used to determine possible sexual dimorphism based on data on standard length and body height for females and males. A χ^2 goodness-of-fit test was performed to compare female to male (F: M) ratios (per month and within size groups), with a hypothesized sex-ratio of 1:1. Differences in size at maturity and size between sexes within length classes were performed using the Mann-Whitney test.

The Kruskal-Wallis test was used to determine differences in gonadosomatic indices among months. Batch fecundity was calculated using the following equation:

BF = nWg/w, in which: BF = batch fecundity, n = number of hydrated oocytes in the sample, Wg = the gonad weight and w = the sample weight. The number of batches was calculated based on the method proposed by Hunter and Macewicz (1985b). The significance level for all analyses was set at $P < 0.05$. The BioEstat 3.0 software program (Instituto de Desenvolvimento Sustentável Mamirauá – Amazonas) was used for these analyses.

Results

Sex ratio and size composition

The French angelfish, *Pomacanthus paru*, is a gonochoristic fish. Differences between sexes were found in total length (Z (U) = 3.1200, P = 0.0018), standard length (Z (U) = 4.2672, P = 0.0000), body height (Z (U) = 2.96, P = 0.0030). Among 236 individuals sexed, 139 were female (58.89%), 86 were male (36.44%) and 11 were undefined (4.66%) (Fig.2). Sex ratios were biased toward females in all bimesters, except September-October. Bimonthly sex ratios were statistically significant only in March-Apr/06 ($\chi^2 = 6.48$, P= 0.01) and January-February/07 ($\chi^2 = 5.12$, P= 0.02), where females were 3.63-fold and 3.25-fold, more abundant than males (Fig. 3). Differences in sex ratios for size classes were recorded only among these length classes [15 - 30 cm (Z (U) = 4.19, P = 0. 0000); 30 - 45 cm (Z (U) = 4.8131, P = 0.0000)] (Fig. 4).

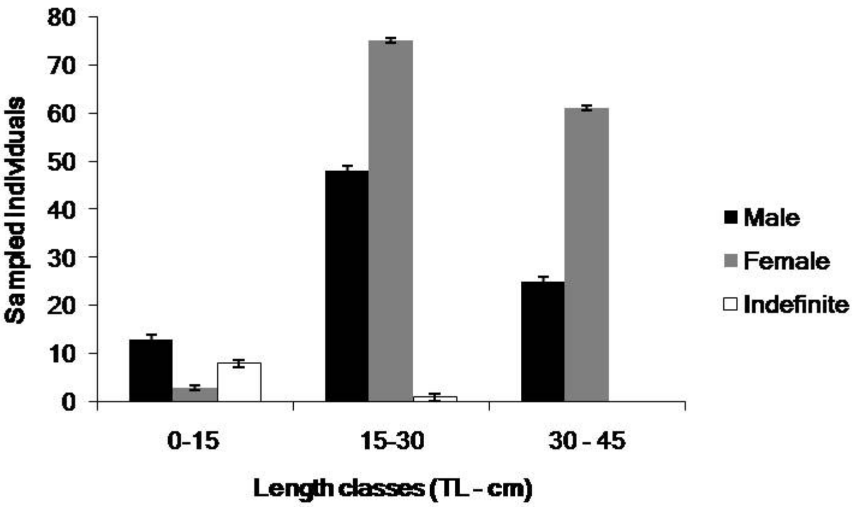


Figure 2: Size distribution between sexes of sampled individuals with bars indicating standard error.

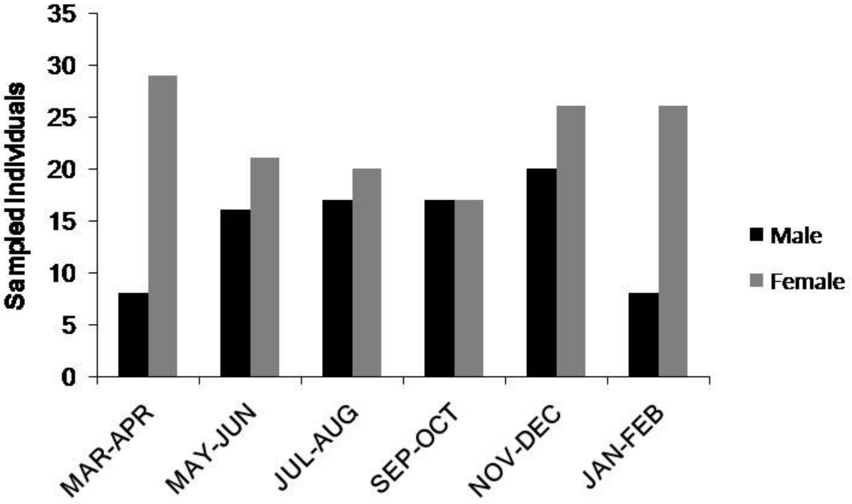


Figure 3: Sampled individuals distribution of female and male of *Pomacanthus paru* among bimesters.

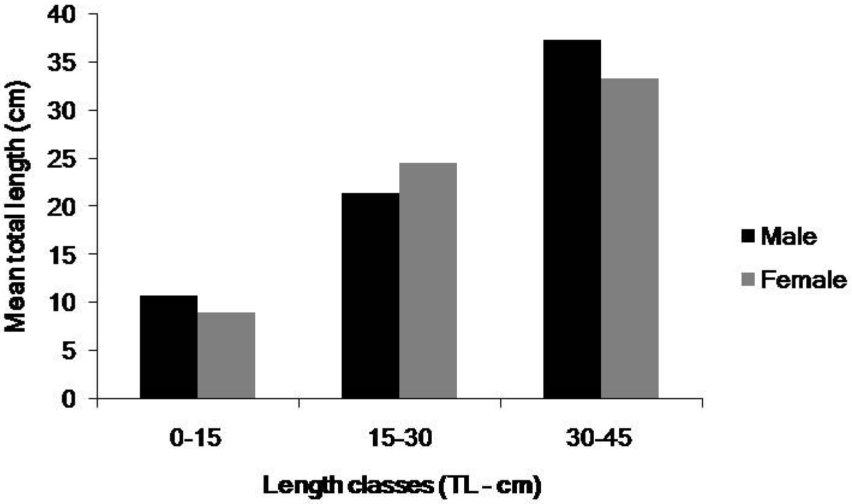


Figure 4: Mean total length distribution of *Pomacanthus paru*.

Spawning season and gonadal development

Monthly changes in mean GSI for males and females are presented in Fig. 5 and were not statistically significant for males (K-W test male H = 4.6852 P= 0.4555), but significant for females in bimesters Mar-Apr and Nov-Dec (K-W test female H = 13.4832 $P < 0.05$). The analysis of GSI for females indicates an increase beginning in January-February, with a peak in March-April, and gradual decline until August. GSI attained high levels in September-October, but declined in November-December and then began to rise. For males, the GSI is the highest in March-Apr, as observed in females. The GSI then declined abruptly and continued nearly the same until March.

The immature females occurred from April to January, but the highest frequency was found between July-August and September-October. Maturing and ripening stages occurred throughout the year, except in June, September, October and December for the maturing stage. The spawning stage was dominant in September-October and from January until April. In this period, the GSI increased, mainly between January and April and in October. Resting ovaries were recorded throughout the year, with the exception of July, September and February. However, the spawning stage was recorded in these months (Fig. 6).

Males in immature stages were only recorded in a few months. The maturing stage was not observed in April, December or February, but spawning and spent stages were recorded. The ripening stage was more frequent in July-August and November-December. From May until October, the spawning stage was dominant. Individuals in the spent stage were recorded throughout the year. Resting testes were not recorded only in March-April and September-October (Fig. 6).

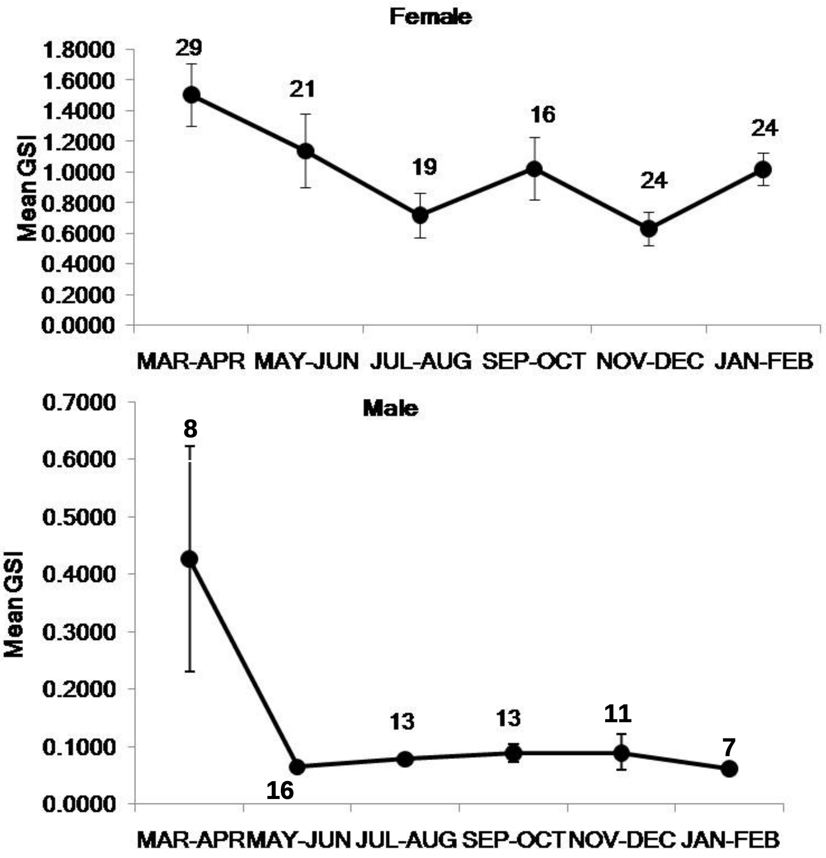


Figure 5: Bimonthly changes of the mean gonadosomatic index (GSI) in female and male *Pomacanthus paru* with bars and values indicating the standard error and the number of individuals analyzed for each stage, respectively.

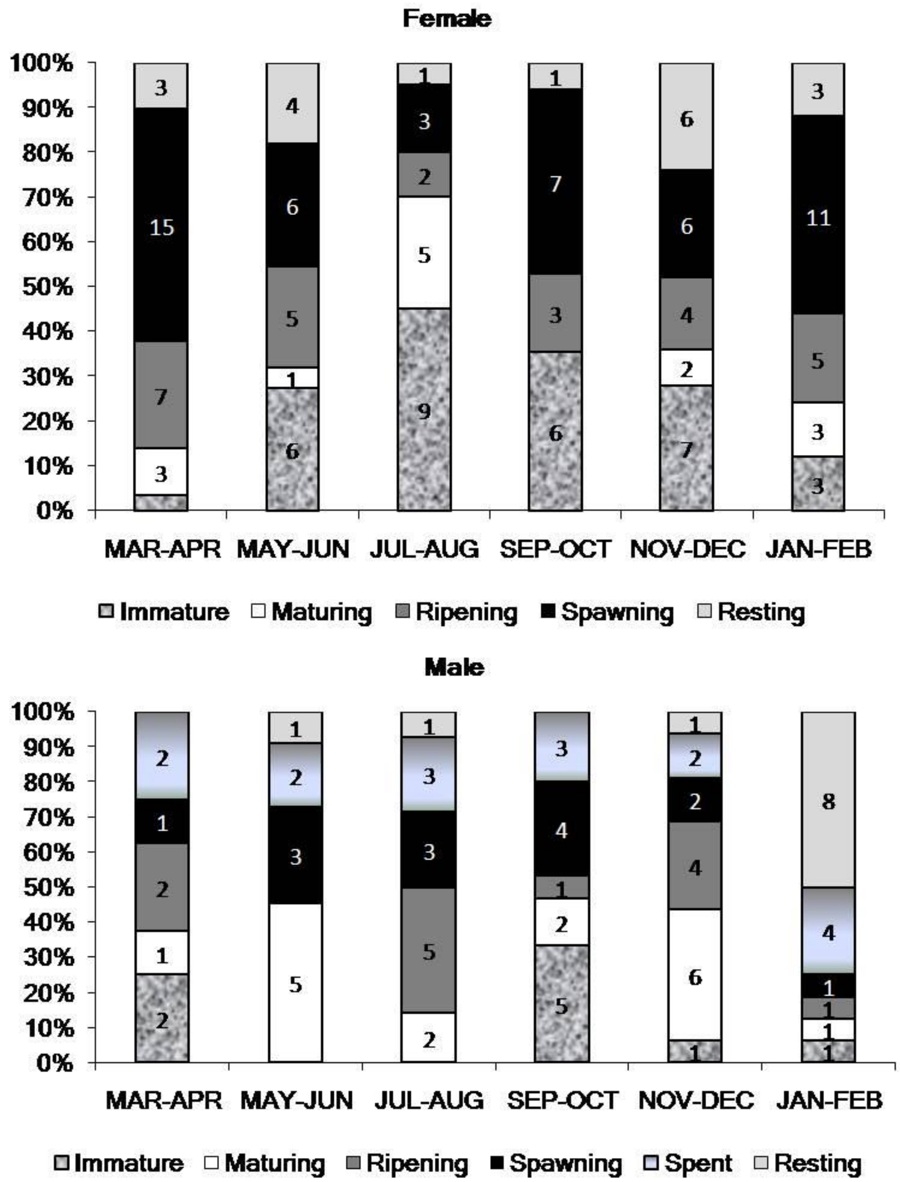
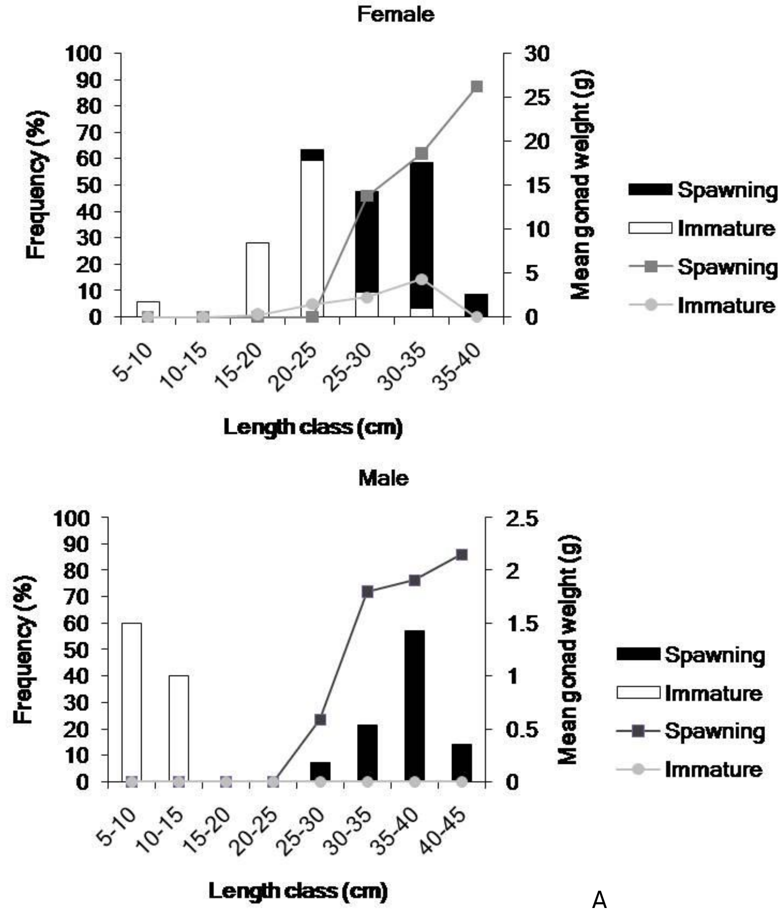


Figure 6: Bimonthly percentage frequencies of occurrence of gonadal developmental stages in female and male *Pomacanthus paru*. The values inform the number individual analyzed for each stage.

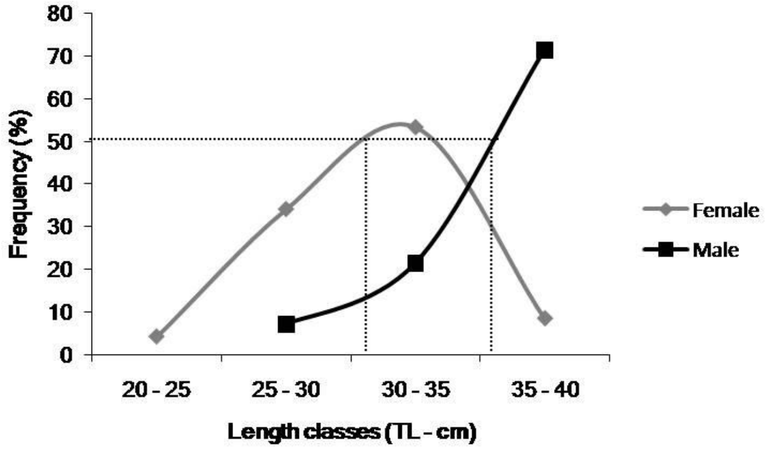
Size at maturity

Individuals fit for reproduction appeared at 20 to 25 cm for females and 25 to 30 cm TL for males. The L50 were different between sexes, being maturity reached at

30cm for females and 35 cm for males (Z(U) = 4.3532 P = 0.0000). The smallest mature female and male were 23.18 cm TL and 25.24 cm TL, respectively (Fig. 7).



A



B

Figure 7: Percentage of individuals in spawning and immature stage versus length class and mean gonad weight for female and male (A) and graphic showing the L_{50} for female and male of *Pomacanthus paru* (B).

Fecundity

Table 2 displays estimates of the minimum, maximum and mean values for the batch and relative fecundities. Hydrating oocytes ranged in size from 350 to 800µm.

Table 2: Minimum, maximum and mean values of batch (BF), total (TF) and relative (RF) fecundities of *Pomacanthus paru*.

	TL* (cm)	BW* (g)	BF	RF
Minimum value	23.18	428	109.6	4.73
Maximum value	34.24	1 354	29 475	860.83
Mean value ± SE			3.751.62 ±1.396.78	118.48 ± 41.42

*TL = total length, BW = body weigth

Discussion

Sex ratio and size composition

In the present study, *Pomacanthus paru* was classified as a gonochoric species. An overlap in size distributions, similar mean sizes of adult males and females and the lack of histological evidence for hermaphroditism support this sexual pattern. Hermaphroditism is a common process in tropical fish families and is recorded in, at least, eighteen families (Ross, 1990). In the family Pomacanthidae, protogynous hermaphoditism has been found in three genera: *Centropyge* (Moyer and Nazakono, 1978; Bruce, 1980), *Geniacanthus* (Bruce, 1980) and *Holacanthus* (Hourigan and Kelley, 1985, Nottingham *et al.*, 2003). However, it has been suggested that *Pomacanthus* species are gonochoristic (Michael, 2004). In most gonochoric fish, size distributions of females and males overlap broadly. However, the population structure alone is not a reliable indicator of sexual pattern, as bimodal size may be observed in gonochoric species that possess size dimorphism (Sadovy, 1986) or differential rates of growth, maturation, or mortality by sex (Sadovy and Shapiro, 1987).

Species that occur in pairs are commonly gonochoric, as was observed for *Pomacanthus paru* in the present study. However, the gonochoric pattern also occurs in fishes with group-spawning mating systems, where all males have access to females, and sperm competition is intense (Warner, 1982). The mating system of the genus *Pomacanthus* depends on the population density in a given location. In some areas, *P. paru* form stable pairs, whereas in locations in which the species is abundant this fish forms harems (Michael, 2004). However, some authors state that *Pomacanthus paru* is consistently found in pairs, regardless of whether the species is abundant or not (Moyer *et al.*, 1983). Despite the common occurrence of pairs, spawning involves pairs and solitary individuals, as observed by Thresher (1980) and Moyer *et al.* (1983).

Sexual dimorphism is uncommon among marine teleost fish. It can be manifested as changes in body shape during growth, which is related to protogynous hermaphroditism. However, in gonochoristic species, size differences between sexes have also been recorded (García-Cagide *et al.*, 2001), as was observed for *Pomacanthus paru*. Sexual size dimorphism is a ubiquitous characteristic of angelfish, for which males are generally larger than females. Size dimorphism and sexual dichromatism have been recorded in some species of the genus *Pomacanthus* (Thresher, 1982). Feitosa *et al.* (unpublished data - chapter 2) observed a different pattern of growth between sexes, in which males continued to grow, while females already reached the asymptotic length. Reinforcing the size dimorphism for *Pomacanthus*, Moyer *et al.* (1983) stated that sexual size dimorphism is not conspicuous in most adult pairs of *P. paru*, however after a close examination, a slight size difference between paired individuals can be noticed.

Sex ratio is a population characteristic that plays an important role in reproduction. In species of snappers, jacks and grunts, females are more abundant in almost all length classes and reach a larger size than males (García-Cagide *et al*., 2001). In these cases, female dominance may be explained by a greater survivorship, but also by differences in habitat preference (García-Cagide *et al*., 2001). According to Aburto-Oropeza *et al*. (2000), the sex ratio of angelfish populations (proportion of sexes and sizes) seems to be determined by the mating system. In the family Pomacanthidae, there are different reproductive styles among genera (Allen, 1979) and most are protogynous hermaphrodite (Thresher, 1984). Few studies, however, have addressed the role of other factors, such as food availability, in the structuring of angelfish communities (Arellano-Martínez *et al*., 1999; Aburto-Oropeza *et al*., 2000).

Aburto-Oropeza *et al*. (2000) observed differences in habitat preference between sexes in the angelfish *Holacanthus passer*, for which a significant habitat overlap was only recorded between large females and small males. For this angelfish, sex differed significantly over the size range, with females prevailing at smaller lengths and males at larger lengths (Arellano-Martínez *et al*., 1999). It may be suggested that there is a habitat preference between sexes in *Pomacanthus paru*, as a similar pattern to that described by Arellano-Martínez *et al*. (1999) was recorded in the samples of the present study. In a study performed in Caribbean waters, Aiken (1983) found that the sex ratios of the various species of chaetodonthids and pomacanthids caught in traps were female biased, mainly in *Pomacanthus arcuatus* (M:F – 1:2.51) and *Pomacanthus paru* (M:F – 1:3.28). The author attributes these variations to differences in sample site depths.

Other explanations for this pattern are that females enter fish traps more readily than males (Gaut and Munro, 1983) or that females fit for reproduction move into the sampling area and are more susceptible to traps, due to their reproductive status. Females are especially more abundant in samples in months related to the spawning peak. As smaller individuals between 0 and 15 cm TL (length class dominated by males) were not caught by traps, the female dominance of *P. paru* may be explained by these factors.

Spawning season and gonadal development

Although female of *Pomacanthus paru* has two reproductive peaks and male one, the species is probably capable of spawning throughout the year, based on the occurrence of females in the spawning stage and males in the spent stage in nearly every month. In Caribbean waters, ripe individuals of *P. paru* have been collected in May, July, August, October and November and from January to August, with a maximal proportion in April for *Holacanthus ciliaris* (Munro *et al.,* 1973). For *Pomacanthus arcuatus***,** the greatest proportions of ripe fishes were collected in October and January, but according to Munro *et al*. (1973), there is no discernible seasonal trend in the data. Ripe individuals of *Holacanthus tricolor* were found in all months, with the exception of December for which there is no data. According to Moyer *et al*. (1983), *H. tricolor* spawns most of the year in Puerto Rico. Arellano-Martínez and Ceballos-Vásquez (2001) also collected ripe fishes of *Holacanthus passer* in April. Although ripe *P. paru* were collected in every month in the present study, the data presented are in agreement with those obtained by Munro *et al*. (1973), Moyer *et al*. (1983) and Arellano-Martinez *et al*. (2001) with regard to spawning months.

The gonadosomatic index value is an indicator of the duration of the reproductive season, but caution should be exercised in its interpretation due to regional and temporal variations (Jons and Miranda, 1997). In fact, seasonal variations in GSI must be analyzed together with other factors, such as the monthly proportion of fish in different stages of gonad development (García-Cagide *et al*., 2001). In the present study, the GSI index demonstrated a unique and common peak (March – April) for both sexes. Two peaks were recorded for females, - a strong peak in January-February and March-April and a weak one in September-October. A high frequency of males in spawning stages was recorded in May until October, when the GSI index values were low. In *Hyporhamphus australis*, some gonads have been found in spawning condition outside their respective GSI-defined peak spawning periods (Hughes and Stewart, 2006). For two species of tropical Atlantic hemiramphids it has been demonstrated that, while the spawning of young females is strongly seasonal, older female fish spawn throughout the year (McBride and Thurman, 2003). Moyer *et al.* (1983) found a daily spawning cycle that occurs throughout all or most of the lunar cycle in six American species and fifteen Western Pacific species of angelfishes. Many tropical fish are intermittent spawners and, for such individuals, the GSI provides information for only a certain period of the reproductive cycle, rather than entire year (García-Cagide *et al*., 2001). Despite the values of this index, the data of the present study support a pattern of reproductive activity throughout the year, with two spawning peaks.

Size at maturity

Size at maturity is influenced by environmental and genetic factors (Wooton, 1979), but fishing pressure can also affect this parameter (Jennings *et al*., 2001). Based on GSI index of *Holacanthus passer*, Arellano-Martinez and Ceballos-Vásquez (2001) affirm that due to the gonad size, male fish requires less stored energy for gonadal development and it may start to mature earlier than females. However, the results of the present study demonstrated that *P. paru* females mature earlier than males. Spawning stage first appeared in females at 23 cm TL and in males at 25 cm TL. Besides, the L_{50} for females was 5 cm smaller than males. The total length of the smallest mature *Pomacanthus paru* female caught in Caribbean waters by Aiken (1983) was 23 cm. The author suggests that males tend to mature at a larger size than females, as no male was captured. The results of the present study corroborate with Aiken (1983).

Fecundity

According to Hunter and Goldberg (1980), the number of hydrated eggs in ovaries prior to ovulation should give the most accurate estimate of fecundity and this method has been used on several *Engraulius* species (Hunter and Goldberg, 1980; Hunter *et al*., 1984; Hunter and Macewicz, 1985a), as well as on the chub mackerel (*Scomber japonicus*) (Peña *et al*., 1986) and dwarf herring (García-Cagide *et al*., 2001). The fecundity obtained in the present study was much lower than that described by Aiken (1983), who found a mean value of 34 200. This difference may be due to the sample size, as Aiken (1983) only analyzed two individuals, whereas 44 females were analyzed in the present study. Among this total, one fish had values similar (29 475) to that determined by Aiken (1983). It may be suggested that the sample size used by Hourigan and Kelley (1985) was insufficient for a comparative analysis. The authors only sampled ten *Holacanthus tricolor* females, - five in May and five in September. The authors recorded a higher number of hydrated oocytes: 15 330 ± 5 490 in May and 35 840 ± 18 480 in September. Similarities were recorded between the present study and that performed by Arellano – Martínez *et al.* (2006) for *Pomacanthus zonipectus*, in which these authors found a mean values of 79.4 ± 9.2 for relative fecundity. The range size of hydrating oocytes found in the present study corroborates the results obtained by Hourigan and Kelley (1985) for *Holacanthus tricolor* (363 – 721 µm) and by Arellano-Martínez *et al.* (2006) for *Pomacanthus zonipectus* (675 ± 1.2 µm).

Besides the sample size issues, the definition of spawning stage without determinate the minimal quantity of these oocytes for this stage should be revised. This definition, based on the simple presence of hydrated oocytes, may be influencing the higher variation recorded in the present results of fecundity.

Considerations

The present study provides important information on the reproductive biology of *P. paru*. The French angelfish is a gonochoristic species. Some hypotheses, such as habitat preference between sexes, greater female susceptibility to trap fisheries and female migration to sampling areas, may explain the role of population sex structure of *Pomacanthus paru*. However, further studies are needed to clear up this issue. This species is mainly targeted by the aquarium trade. Species-based quotas have been established by the Brazilian environmental protection agency (IBAMA) to ensure that harvests are maintained at a sustainable level. Despite the determination of the size at maturity in the present study, this information is not relevant for the aquarium trade management, as most commercialized ornamental fish are juveniles under 10 cm in standard length. It can be suggested that conservationist issues should be based on quantitative data (capture) per area and the establishment of protected areas.

Pomacanthus paru is also commonly caught as bycatch in trap fishery, an unregulated multi-specific activity. Specimens captured as bycatch are not landed and are usually returned to the sea (many still alive, according to the fishermen). The survival of these individuals is doubtful, as the traps are hauled quickly to surface and problems related to decompression have often been reported. Little is known regarding the impact of this activity, being difficult to manage it. Monitoring and management of this fishery, a growing activity with catches exported to the international market, is mandatory in order to assess the impact of this kind of fishery on both target and non-target species.

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Capítulo 2 - **Idade e crescimento do Paru preto *Pomacanthus paru* (Teleostei: Pomacanthidae) no nordeste do Brasil.**

Chapter 2* - **Age and growth of the French angelfish *Pomacanthus paru* (Teleostei: Pomacanthidae) in Northeastern Brazil.**

* Capítulo em formato de artigo e padronizado de acordo com as normas da revista *Journal of Fish Biology.*

Age and growth of the French angelfish *Pomacanthus paru* (Teleostei: Pomacanthidae) in Northeastern Brazil.

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Abstract

The aim of the present study was to describe age and growth of the French angelfish *Pomacanthus paru.* Age was determined by sectioned otoliths alone. All treated otoliths with tetracycline were one year of age and revealed a clear fluorescent mark when observed under UV light. Based on age determination studies, increments observed in sagittae otoliths of tropical fish may be consistently interpreted as having annual periodicity. Otolith weight increased exponentially with standard length and linearly with age. This indicates that otolith growth continues with age and is independent of size. Age of the fish in the sample ranged from 1 to 27 years, with males exhibiting lower K and greater asymptotic length than females. *P. paru* attains maximal size slowly and has a long lifespan. Most linear growth is achieved by approximately 52% of lifespan. Besides being an important ornamental species, *P. paru* has been commonly caught as bycatch in trap fisheries for decades. These growth parameters should be used with the purpose of managing fisheries targeting this species before more meaningful limits can be imposed. In the case of aquarium trade management, it can be suggested that conservationist issues should be based on capture per area and the establishment of protected areas.

Key words: *Pomacanthus paru*, growth parameters, validation, aquarium trade.

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Introduction

One of the most commonly employed methods for age determination is the interpretation of rhythmic deposition in calcified tissues used as time markers (Casselman, 1987). Considered the most appropriate structure for age determination (Six & Horton, 1977), otoliths are calcium carbonate concretions in aragonite crystal form (Degens *et al*., 1969) and are found in the membranous labyrinth of the inner ear of teleost fish (Lowestein, 1971). The use of otoliths for age determination was first developed for temperate fish populations (Brothers, 1980), which have seasonally controlled periods of growth and spawning. Due to the lack of seasonality in tropical waters, the use of the otolith method for age studies on tropical fish began as a complex process. However, in the late 1980s, Longhurst & Pauly (1987) stated that the growth of tropical fish follows expected seasonal patterns, which can be observed using length frequency data or the analysis of seasonal bands in otoliths.

Despite the importance of studies on age, growth, mortality and longevity, limited information is available on the population dynamics of tropical fish (Radtke, 1987). Age and growth studies are crucial to understanding the life history traits of fish species, including population structure, changes in population growth due to environmental variations and recruitment success (Dee & Radtke, 1989; Zekeria *et al*., 2000). Such studies are required to explain demographic characteristics that are unique to tropical fish populations and determine environmental influences on life history events (Radtke, 1987). Furthermore, growth and ageing studies on species undergoing fishery pressure provides indispensable information for stock assessments and fishery management (Aliaume *et al*., 2000).

Information on growth of coral reef fish is limited to a few families, such as acanthurids, haemulids, lutjanids, scarids, serranids, sparids (Choat & Axe, 1996; Choat *et al*., 1996). For the family Pomacanthidae, estimates from age-at-length data are only available for one species – *Pomacanthus imperator* (Bloch, 1787) (Chung & Woo, 1999). Moreover, the method employed for ageing this species was the reading of scales, which is prone to error (Williams & Bedford, 1974; Erickson, 1983).

Fish from the family Pomacanthidae are among the most colorful and recognizable groups of reef fish (Thresher, 1982). This family comprises 88 species, consisting of eight genera and represents one of the most noticeable components in reef fish assemblages, with representatives in all tropical seas (Allen *et al*., 1998). Although angelfish are analogous in overall appearance, there is a diverse range of ecological traits within the family, such as differences in body size, color pattern, reproductive system and diet (Bellwood *et al*., 2004).

The French angelfish *Pomacanthus paru* (Bloch, 1787) occurs from Florida, the Bahamas and the northern Gulf of Mexico down to Brazil (Menezes *et al*., 2003). It is also recorded for St. Peter and St. Paul’s Rocks (Feitoza *et al*., 2003) and Ascension Island (Maugé, 1990) in the Central Atlantic and the Gulf of Guinea. Maximal length reported for this species is 41 cm (Cervigón, 1993). It is distributed in waters from 5 to 100 m, with juveniles and subadults being more frequent in shallow waters. This species is an omnivore and sponges are its main food item (Randall, 1967). It is a gonochoric species (Feitosa *et al*., unpublished data) and it seems that adults generally form stable pairs, although in areas where the fish is more abundant, it is known to form small harems (Thresher, 1984; Michael, 2004).

Pomacanthid species are among the most harvested for the Brazilian aquarium trade and *P. paru* is one of the most exported species (Nottingham *et al*., 2000; Araújo & Albuquerque-Filho, 2005; Monteiro-Neto *et al*., 2003). Along with the fishing pressure by the Brazilian aquarium trade, this species is also caught as bycatch (Feitosa *et al*., 2008). Management measures for this species are restricted to a Federal Act (56/04) established in 2004 with the purpose of managing the aquarium trade through the establishment of export quotas.

In the present study, age and growth of *P. paru* were studied through the validation and examination of annual marks in sagittae otoliths.

Materials and methods

Sampling design

The fish were sampled from commercial boats from the Itamaracá Island Fishing Colony, located on Pilar beach (7°45’17.80’’S – 34°49’26.46’’W) (Itamaracá, state of Pernambuco, Brazil – Fig. 1). These fish were caught as bycatch during trap fishery activities targeting the spotted goatfish, *Pseudupeneus maculatus* (Bloch, 1793), and parrotfish, *Sparisoma* spp. In this fishery, traps are deployed unbaited next to reefs and hauled 6 hours later. Occasionally, depending on factors such as wind and currents, some traps are left for longer periods and only hauled after 48 hours. Sampling was performed weekly from March 2006 to February 2007. As individuals smaller than 15 cm are not captured in this type of fishery, such individuals were bought from fish collectors between September 2006 and February 2007.

Figure 1: Location of sampled site.

Otolith ageing

Fish were measured for total length [TL (cm)] and standard length [SL (cm)], weighed (g) and sexed. The largest otoliths (*sagittae*) were removed for analysis and stored dry. Whenever possible, the left otolith was used for both whole and sectioned views. Rings were counted for whole otoliths by immersing the otoliths concave side up on a watch glass with a blackened bottom containing 100% ethanol and viewed through a binocular dissecting microscope. Opaque bands were counted on the distal surface from the nucleus to the dorsal side. Prior to sectioning, all pairs were measured and weighed to the nearest 0.01 g.

The otoliths were embedded in an epoxy resin prior to reading. Each otolith was sectioned transversely (approximately 0.3 mm thick) with a low-speed saw. Sections were mounted on microscope slides with Histomount mounting media and examined for opaque bands under a dissecting microscope using reflected light.

To assess the precision of the readings, two independent readers counted age marks on whole and sectioned otoliths with no knowledge of fish size. A second reader tested the precision of the first reader for a subsample of 50 otoliths sections (21%). With these results, the index of average percentage error (IAPE) between readers was calculated (Beamish & Fournier, 1981). If the readers diverged by more than 10% for a given otolith section, the readings were repeated. The data were included in the analysis only when the IAPE was below 10%.

A validation experiment was performed to determine the accuracy of the otolith readings. For this test 6 individuals were used. As individuals sampled for this test were smaller than 6 cm, the specimens were marked by immersion in a solution of tetracycline and seawater (Beamish & McFarlane, 1987), with an initial concentration of 100 mg l^{-1}. Each subsequent four hours, the same concentration (100 mg l^{-1}/h) was added to the solution until reaching the maximal concentration of 500 mg l^{-1}. The fish were kept in this solution for 12 hours (Hernaman *et al.*, 2000). After this procedure, they remained in aquarium for 1 year, with a mean salinity and temperature of 30 $\%_0$ and 28°C, respectively.

The treated otoliths were sectioned in the same way as the unlabeled ones and analyzed under a microscope with an ultraviolet light source to detect the fluorescent mark. An external fiber-optic light was coupled to the microscope in order to observe the opaque bands and fluorescent marks simultaneously (Ferreira & Russ, 1992).

Growth was investigated by fitting the von Bertalanffy (1938) growth function to size-at-age data using the nonlinear optimization method on the Kaleidagraph 4.0 software program. The model was fit for each sex separately. The growth function is defined as follows:

$$Lt = L\infty \left(1 - e^{-k\,(t\,-\,t0)}\right)$$

in which Lt is length at time t, L∞ is asymptotic length, k is the growth coefficient and t_0 is the hypothetical time at which length is equal zero. The rate of increase in size with age between sexes was compared using the Mann-Whitney test ($\alpha = 0.05$). The paired t–test was used to compare the weight of left and right sagittae otolith ($\alpha = 0.05$). The relationship between otolith weight, standard length and age was determined through simple linear regression analysis.

Parameters of the length-weight relationship were obtained by fitting the function $W= aL_s^b$ to length and weight data, in which W is the total weight, a is the constant (determined empirically), L_s is the standard length and b is close to 3 for species with isometric growth.

Results

In French angelfish, the sagittae otolith is the largest and oval shaped, with a pointed rostrum. It is laterally compressed, with a heterosucoid sulcus acusticus (Fig. 2). A pattern of opaque and translucent bands can be recognized and counted on whole and sectioned otoliths. Nevertheless, counting opaque bands on whole otoliths is a complex task due to the overlapping of bands at the margins in larger fish and regular divergence in counts between different otolith regions, mainly in the dorsal area. Due to these complexities, the age of *P. paru* was determined only on sectioned otoliths (Fig. 2).

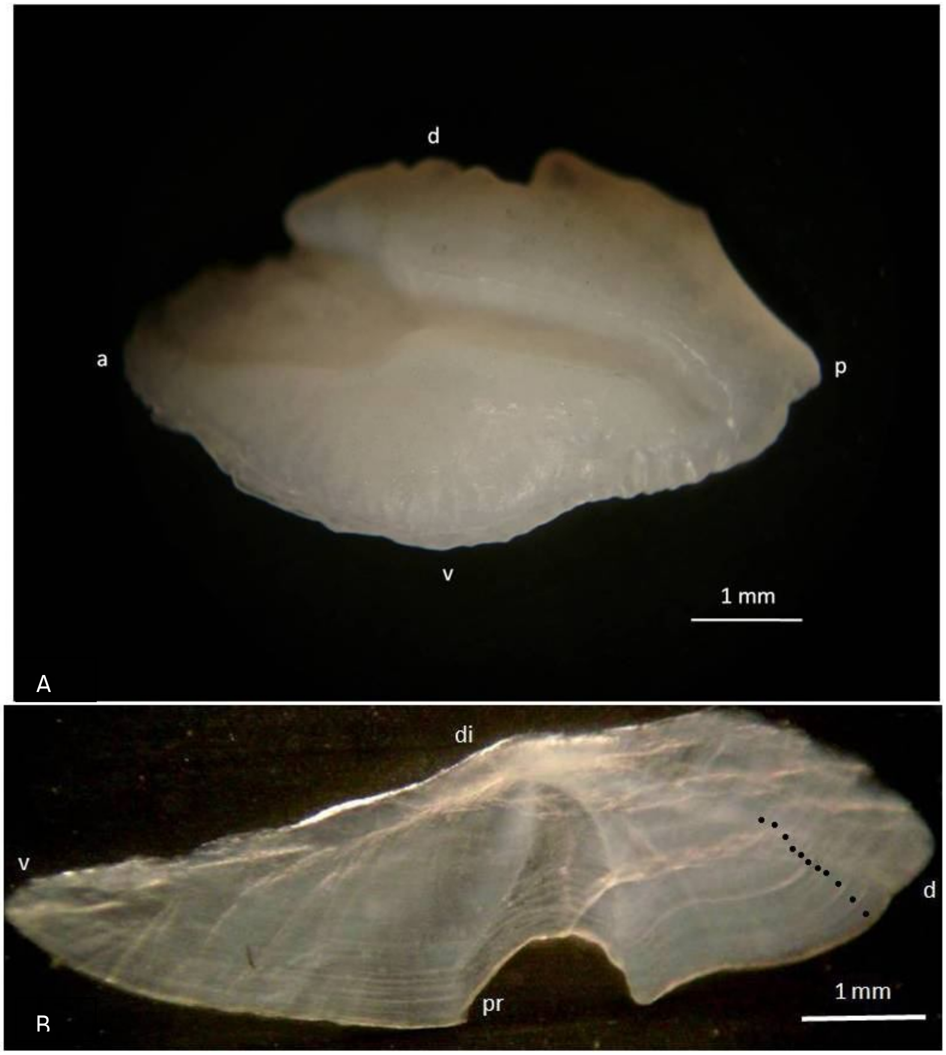

Fig. 2: Whole (A) and sectioned (B) *sagittae* otoliths of a 8 and a 11 years old *Pomacanthus paru* (a = anterior, p = posterior, d = dorsal, v = ventral, di = distal, pr = proximal).

The sections of all treated otoliths revealed an opaque band between a clear fluorescent mark and the edge when observed under an external light source and UV light (Fig 3). Observations from all of the sections support the hypothesis that the annulus is formed once a year. All the treated French angelfish were one year old.

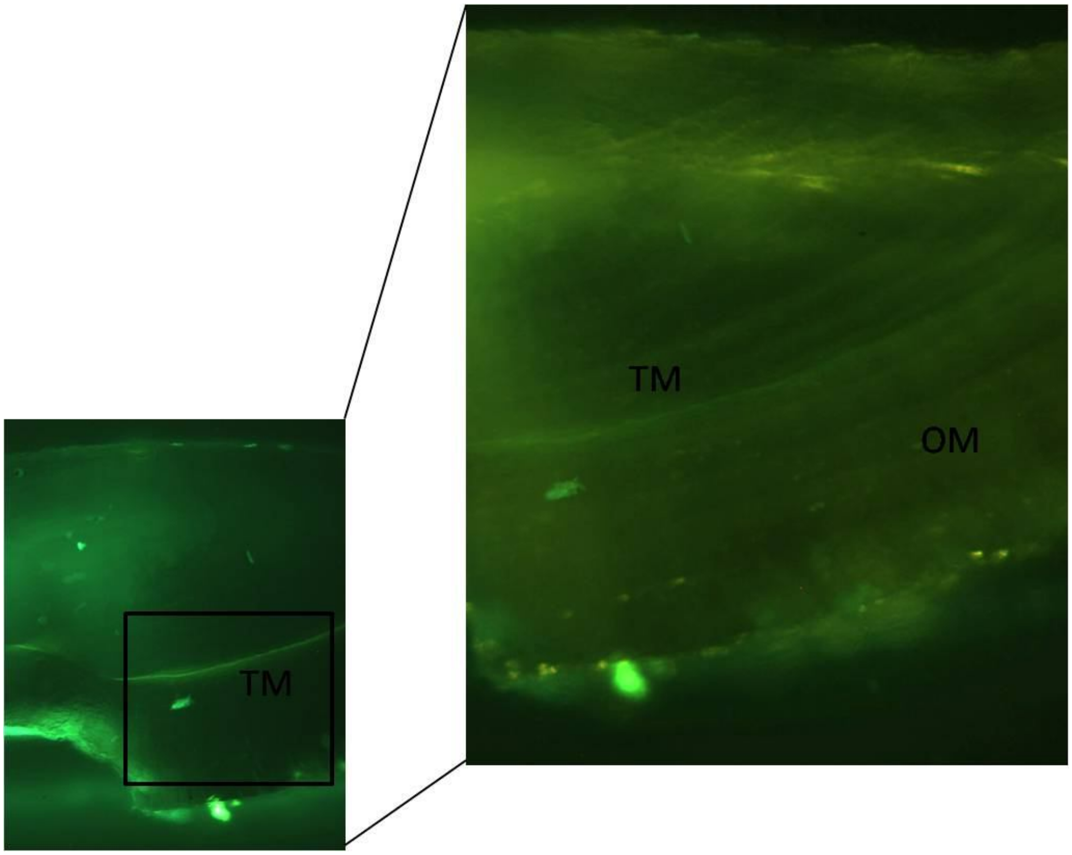

Fig. 3: Sectioned otolith treated with tetracycline observed under UV light (A) and under an external light source and UV light (B). TM – tetracycline mark, OM – opaque mark.

Otoliths ranged in weight from 0.002 (7.81 cm, 16 g, 4-year-old fish) to 0.169 g (38.64 cm, 1.396 g, 26-year-old fish). There was no significant difference between the weight of the left and right otolith (paired t-test, $n = 153$, $P > 0.05$). The exponential relationship between otolith weight and standard length follows the equation $OW = 0.0049e^{0.1082\ SL}$ ($r = 0.89$, $n = 184$), in which OW is otolith weight (g) and SL is standard length (cm) (Fig. 4). Otolith weight increased linearly with age and was described by the equation $OW = 0.0053\ SL + 0.0036$ ($r = 0.64$, $n = 167$) (Fig. 5). The relationship of total weight to standard length is $W = 0.1192SL^{2.794}$ ($r = 0.98$, $n = 236$) (Fig. 6).

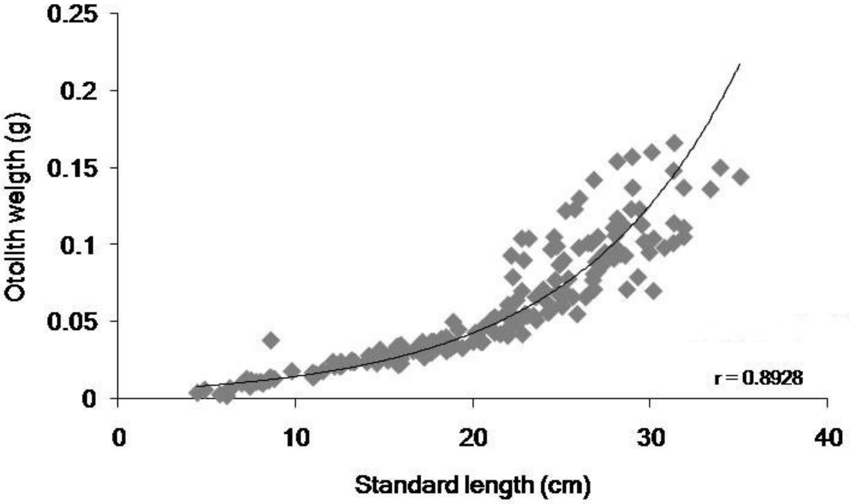

Figure 4: Relation between *sagittae* otolith weight and standard length for *Pomacanthus paru*. SL = standard length (cm), OW = otolith weight

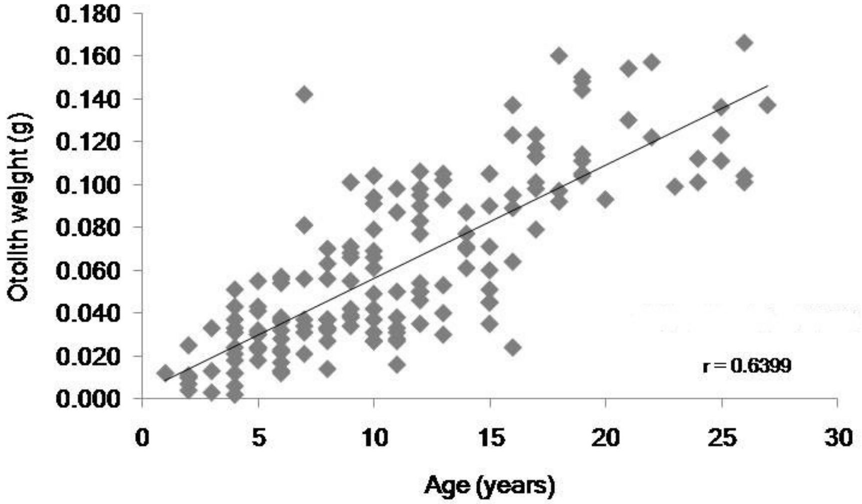

Figure 5: Relation between otolith weight and age for *Pomacanthus paru*. A = age years, OW = otolith weight.

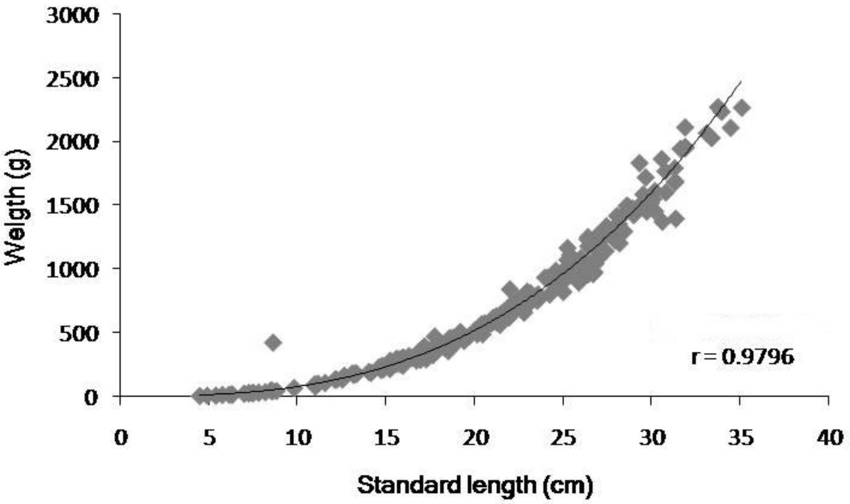

Figure 6: Relation of weight to length for *Pomacanthus paru*. SL = standard length (cm), W = total weight (g).

Age of the specimens in the sample ranged from 1 to 27 years (Fig. 7). Females age range was 2 to 27 years and males was 1 to 26. Statistically differences were recorded for size at age between sexes only for the age ranging from 16 to 20 (n = 27) and 21 to 27 years (n = 15) (Mann-Whitney test, $P < 0.05$). A total of 2.30% of the otolith sections were unreadable and were not included in the analysis. The average percentage error between readers was low (9.80%). French angelfish are long-lived (to > 27 years) and attain maximal size slowly, especially males. The plots were fitted best by the von Bertalanffy growth function for females: $Lt = 26.66\ (1 - e^{-0.18\ (t\ +0.56)})$ and males $Lt = 44.11\ (1 - e^{-0.05\ (t\ +3.34)})$.

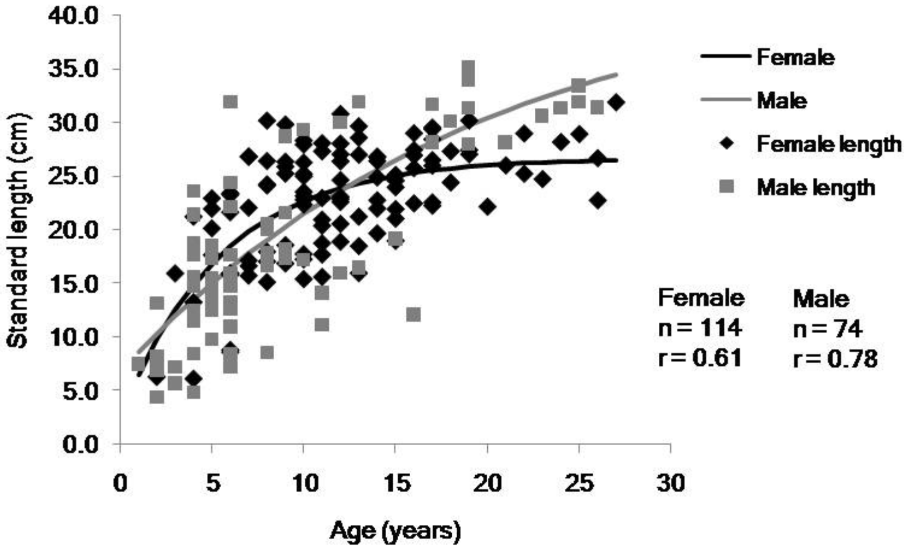

Figure 7: Von Bertalanffy growth curve fitted to length at age data of female and male of *Pomacanthus paru*.

Discussion

Despite being widely recognized as important coral reef inhabitants and very significant in marine aquarium trade, there are few studies on age and growth of representatives of the family Pomacanthidae (Chung & Woo, 1999). This may be attributed to the difficulty in sampling individuals in all length classes.

In the present study, sagittae otoliths – which are commonly the largest (Panella, 1971) – from French angelfish exhibited a regular pattern of annual opaque and translucent bands, the readings of which were highly repeatable, with an average error of 9.80%. Based on age determination studies, increments observed in the sectioned sagittae otoliths of tropical fish may be reliably interpreted as having annual periodicity (Choat & Robertson, 2002).

The relationship between otolith weight and age was linear. This suggests that otolith growth is continuous for *P. paru*, while fish size (standard length) is asymptotic at intermediate ages, indicating that this relationship is not continuous. Thus, otolith growth is continuous with age and is independent of fish size. It seems that this is a general rule of fish growth, as this pattern has been observed in several other species (Dee & Radtke, 1989; Hart & Russ, 1996; Schwamborn & Ferreira, 2000; Amezcua *et al.*, 2006; Araújo & Martins, 2006; Zekeria *et al*., 2006). Bands were deposited annually and the weight of otoliths increased throughout the lifespan, thereby fulfilling the fundamental criteria required for ageing (Fowler & Doherty, 1992).

Growth data for the French angelfish fitted the von Bertallanfy model well. Longevity was up to 27 years and maximal asymptotic standard length was 44.11 cm, with males exhibiting lower K and greater $L\infty$. However, the longevity was nearly the same between sexes. Females reach asymptotic length nearly at age 15 and in this age males continue to grow attaining greater sizes at the subsequent ages. The samples used in the present study came from trap fisheries and, consequently, were related to the selectivity of this fishing gear. For the angelfish *Holacanthus passer* sex ratio differed significantly over the size range, with females prevailing at small lengths and males at larger lengths (Arellano-Martínez *et al.* 1999). A similar pattern was observed in the present study, in which males were larger than females. It is possible that when females attain age at sexual maturity, they strongly reduce their growth.

Reinforcing the differences in growth patterns between sexes, Feitosa *et al.* (unpublished data - chapter 1) observed size dimorphism in *Pomacanthus paru,* with males attaining larger sizes. Studying *Pomacanthus paru* in the Caribbean (St Croix, US Virgin Islands), Aiken (1983) states that males tend to mature later than females. This difference in size at maturity was also recorded by Feitosa *et al.* (unpublished data - chapter 1). These authors affirm that individuals fit for reproduction appeared at 20 to 25 cm for females and 25 to 30 cm TL for males, with L50 different between sexes, being maturity reached at 30cm for females and 35 cm for males. These findings corroborate with the present results, in which males presented lower K and had greater asymptotic length when compared to females.

Using size distributions of trap catches of the Gray angelfish, *Pomacanthus arcuatus* (Linnaeus, 1758) based on moderate samples from the unexploited sites of Pedro Bank and the Porto Royal reefs, Aiken (1983) determined that 60 cm TL is the asymptotic length. Although *P. paru* and *P. arcuatus* are sympatric species, maximal length ($L\infty$) was divergent, even considering that the $L\infty$ established for *P. arcuatus* was based on total length. Aiken (1983) also examined scales from *P. paru* and found that rings or marks were present, but no interpretable pattern was discernible. The age and growth study of *Pomacanthus imperator* demonstrated that the $L\infty$, based on standard length, for this specie is 41.19 cm (Chung & Woo, 1999). This value is very large when compared with the $L\infty$ recorded for *Pomacanthus paru* in this research.

Disparity between sets of growth parameters may be attributed to factors such as sample size, sample composition and the method employed to estimate growth parameters as well as divergences in population structure of different locations (Choat & Robertson, 2002; Araújo & Martins, 2006; Grandcourt *et al.*, 2006). Some hypotheses may explain these differences, such as the method used by Aiken (1983) and Chung & Woo (1999), which was length-frequency distribution in the former and the reading of scales in the latter. Another explanation is that *P. paru* occurs in the Atlantic, whereas *P. imperator* occurs in the Central and Indo-West Pacific (Allen *et al.*, 1998). It is likely that differences in environmental (such as different water temperatures) and genetic characteristics can explain the contrast in growth parameters (Grandcourt *et al.*, 2006). Due to the issues mentioned above, Laurec & LeGuen (1981) state that growth parameters are merely simple mathematical supports and have no value as biological characters *a priori*.

P. paru attains its maximal size slowly and has a long lifespan, with most linear growth achieved by approximately 52% of the lifespan. This growth pattern is shared by other reef fish (e.g. serranids, haemulids and lutjanids), which have K values ranging from 0.10 to 0.22 and maximal ages ranging from 9 to 16 years (Matheson *et al*., 1986; Munro & Polunin, 1997). However, *P. paru* lives up to 10 years more and, in the case of males, the K value was smaller than that cited in the literature (Matheson *et al*., *op. cit*; Munro & Polunin, *op. cit*). The low K value and long life among demersal fish suggests that these characteristics are associated to evolutionary success in the reef environment (Huntsman, 1981). According to Matheson *et al*. (1986), long life and relatively large body size maximize gamete and zygote production and allow reef fish to overcome the apparently low possibility of the pelagic offspring encountering a favorable habitat. Conversely, Cuban coastal reef fish, such as haemulids and lutjanids, have a short lifespan and a relatively high growth rate, with species reaching between 35 and 50% of maximal size in the first year of life, according to Claro & García-Arteaga (2001). The authors suggest that these characteristics are related to adaptive mechanisms for avoiding predation on juveniles. Studying the coral trout *Plectropomus leopardus* (Lacepède, 1802) from the Great Barrier Reef, Australia, Ferreira & Russ (1994) recorded a different survival mode in growth. This serranid is relatively short-lived. The growth rate becomes asymptotic from age six onwards and a substantial proportion of total growth is attained early in its life. This rapid growth is favored due to the availability of potential prey and avoidance of predators with the increase in body size. A similar pattern has been observed among scarids and acanthurids from the Great

Barrier Reef, for which growth patterns are highly deterministic and most linear growth is reached in the initial 10 to 20% of lifespan (Gust *et al.*, 2002).

P. paru and other pomacanthids are heavily exploited by the aquarium trade (Moenick, 1991; Wood, 2001a; Monteiro-Neto *et al.*, 2003). The family Pomacanthidae alone comprises 46% of exports by value (Pyle, 1993) and more than 21 species are harvested (Wood, 2001a). The French angelfish is a very important species in the Brazilian aquarium trade and is the second most exported species (Nottingham *et al.*, 2000; Monteiro–Neto *et al.*, 2003; IBAMA, 2007). In the USA (state of Florida), this species is managed through size regulations (Wood, 2001b). In Brazil, a Federal Act (56/04) was established in 2004 with the purpose of managing fishery activities targeting this species through the establishment of export quotas. The extended growth and late sexual maturity (≈ 23 cm TL (female) - Feitosa *et al.*, unpublished data; ≈ 11 years old; 25 cm TL (male) - Feitosa *et al.*, unpublished data; ≈ 15 years old) of *P. paru* and other pomacanthids are angelfish characteristics that will render them vulnerable to extinction (Roberts & Hawkins, 1999). As large-body specimens require a long time to grow, the removal of these specimens through predation or human activities is least likely to be recovered quickly (Chung & Woo, 1999). Individuals of *P. paru* and other species collected for the aquarium trade are generally under 10 cm in standard length and rarely reach as much as 20 cm, which represents juveniles and young adults that have likely not reached sexual maturity (Wood, 2001b).

Chung & Woo (1999) affirm that it was fortunate that the commercial exploitation of *P. imperator* has only focused on the aquarium trade. As individuals longer than 25 cm (SL) are rarely exploited and specimens up to 29.6 cm (SL) are still reproductively active, these fish constitute an important breeding stock if younger individuals are overexploited. However, this is not the case with *P. paru*. Besides the impact from the ornamental fish trade, this species has been commonly caught as bycatch in trap fisheries for decades (Aiken, 1983; Feitosa *et al*., 2008). In this kind of fishery, more than 70% of individuals caught are fit for reproduction (Feitosa *et al*., 2008). Thus, it is possible that this species suffers fishing pressure at all length ranges and that this impact may threaten recruitment to the spawning stock. Besides, *P. paru* presents ecological importance. This species feeds on sponges and zoanthids (*Palythoa*) which compete with corals making their growth difficult.

In conclusion, the results demonstrate that it is possible to obtain estimates of growth for tropical species based on conventional age determination methods. These estimates should be used with the purpose of managing the fishery activity targeting this species. There are some management measures in place for the species throughout its range of distribution, which include size regulation (USA) and species-based exportation quotas (Brazil), all directed to the aquarium fish trade. While these regulations can clearly play an important role in helping to conserve stocks and prevent overexploitation, management measures should also include measures directed to other fisheries. In Brazil the species is commonly captured as bycatch in trap fisheries, an unregulated multi-specific fishery. Ornamental fish species are an important part of the bycatch (Feitosa *el al*., 2008). Those species are caught and usually returned to the sea - many still alive, according to the fishermen. Survival of these individuals, however, is doubtful, as the traps are hauled quickly to surface and problems related to decompression have often been reported.

Monitoring and management of this fisheries, a growing activity with catches exported to the international market, is mandatory in order to assess the impact of this kind of fishery on both target and non-target species. This paper contributes to the knowledge on life history traits of *P. paru*, such as growth rates and age at sexual maturity. Both are minimal requirements for effective fishery management regarding a given species. In the case of aquarium trade management, where most commercialized fish are juvenile and under than 10 cm in standard length, it can be suggested that conservationist issues should be based on quantitative data (capture) per area and the establishment of protected areas.

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Capítulo 3 - **Variabilidade do ADN mitocondrial em populações simpátricas das espécies ornamentais recifais do gênero *Pomacanthus* (Actinopyterigii: Pomacanthidae) no Atlântico Ocidental.**

Chapter 3* - **Mitochondrial DNA variability in sympatric populations of the ornamental reef fish *Pomacanthus* (Actinopyterigii: Pomacanthidae) in the southwestern Atlantic.**

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* Capítulo em formato de artigo e padronizado de acordo com as normas da revista *Journal of Heredity.*

Mitochondrial DNA variability in sympatric populations of the ornamental reef fish *Pomacanthus* (Actinopyterigii: Pomacanthidae) in the southwestern Atlantic

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Running title: Mitochondrial DNA in populations of *Pomacanthus*

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Abstract

A number of studies have shown that reef fish populations have distinct color patterns, but these patterns are not associated with mt-DNA haplotypes. The aim of this study was to analyze the genetic diversity of *Pomacanthus paru* and *P. arcuatus* among populations of each species collected along the coast of northeastern Brazil. Thirty individuals were collected from coasts of Ceará, Pernambuco and Alagoas states. A region of the 16S rDNA was amplified. For phylogenetic analyses additional sequences from four species of the genus *Pomacanthus* were obtained in GenBank. Six mitochondrial DNA haplotypes were found for *P. paru* and a single haplotype for *P. arcuatus.* Topologies indicate that *P. paru* haplotypes are monophyletic and *Pomacanthus* species constitute a monophyletic group. The single haplotype of *P. arcuatus* was shared by individuals sampled in Pernambuco, Ceará (northeastern coast of Brazil) and the Caribbean. The considerations presented here represent a first step toward understanding the population structures of the sister species *Pomacanthus paru* and *P. arcuatus.* Complementary studies on larval settlement, physiological characteristics and anthropogenic actions directed at these populations are needed for a greater understanding of the dynamics of these species.

Keywords: mt-DNA, haplotypes, diversity, *Pomacanthus paru, Pomacanthus arcuatus*

Introduction

Biodiversity based on morphology alone may considerably underestimate evolutionary lineages, particularly when morphological stasis persists after speciation events. This results in species that continue to diverge genetically in the absence of morphological differentiation, producing cryptic or sibling species (Knowlton 1986). Our inability to recognize cryptic species with traditional morphological characters has hindered our understanding of ecological and evolutionary processes. The negative consequences include underestimation of true levels of species-richness, overestimation of potential for long-distance dispersal in supposedly cosmopolitan species, failure to recognize cryptic biological invasions, and misinterpretation of ecological data (Knowlton et al 1992; Geller et al 1997; Rocha-Olivares et al 2001). Even when changes in allele frequencies and differences in sequence data among populations are not considered sufficient for attributing species-level status, organism and population genetic changes must represent the first steps linking micro-phylogeny with macro-phylogeny, because population genetics and phylogenetic history must in truth be parts of a temporal continuum (Avise 2009).

Most marine habitats have traditionally been thought to be highly interconnected by gene flow, because of the absence of obvious barriers to migration and because many marine taxa have pelagic larval stages that have been considered passive dispersers (Palumbi 1994). In contrast, high levels of biodiversity in marine systems suggest that genetic divergence and eventual speciation may be common and rapid in marine systems, indicating that marine biodiversity is actually much higher than earlier estimates (Mathews 2006).

Molecular genetic technologies have changed the way we describe and catalogue biological diversity. Mitochondrial sequence data has sometimes indicated no genetic structure within species (McMillen-Jackson and Bert, 2003), but usually points to an increasing diversity of lineages, from two- to nineteen and perhaps more genetic or specific lineages (e.g., Rocha-Olivares et al 1999; Bichain et al 2007; Rocha et al 2008; Mathews and Anker 2009). When mitochondrial DNA differences become correlated with nuclear DNA sequence patterns, differences in geographical distribution, color distinctions, ecological and behavioral data, or even slight but constant morphological differences, such congruent evidence has be used to establish the presence of distinct evolutionary lineages (Mathews et al 2002; Sandoval-Castillo et al 2004; Thompson et al 2005) .

Phylogeography, the geographical study of population genetics, is now a burgeoning discipline that is establishing a conceptual bridge between microevolutionary population genetics and macroevolutionary phylogenetics and historical biogeography (Avise 2009). These studies have the power to yield insights into the past as well as present evolutionary processes, having been used to uncover information about the roles of geographical history and ecology in generating marine species diversity (e.g., Williams and Reid 2004).

Phylogeography has triggered a renewed interest in speciation processes affecting Neotropical coral reef fish (Taylor and Hellberg 2005; Rocha and Bowen 2008). Reef fish are characterized by their bright and diversified coloration (Sale 1991). Many polytypic species are the result of fragile diagnoses and may include subspecies (Wiley 1981).

Comparing Brazilian populations of two species of reef fish that inhabit the Rocas atoll and the coast of the state of Ceará, Freitas et al (2003) differentiated fish stocks in *Acanthurus chirurgus*, but found unrestricted gene flow among the populations of *Cephalopholis fulva*. The majority of marine organisms are dispersed in the form of pelagic larvae that travel long distances by way of ocean currents. Upon reaching adulthood, many reef fish are benthic, territorial and remain associated with reef substrates (e.g. Sale 1980; Doherty and Williams 1988).

Species of the genus *Pomacanthus* occur in tropical and subtropical waters around the world (Allen et al 1998). Among the thirteen species of this genus, only *P. paru* and *P. arcuatus* occur in the Western Atlantic (Allen 1979; Allen et al 1998; Menezes et al 2003). There is agreement that these species generally share the same habitat and their home ranges overlap (Moyer et al 1983; Hourigan et al 1989). However, authors disagree with regard to the behavior between the two species. Moyer et al (1983) have recorded aggressive postures, whereas Hourigan et al (1989) found no agonistic behavior between the two species. Sympatric sister species such as *P. paru* and *P. arcuatus* can form hybrids and, although there is no record of a wild hybrid between these species (Deloach 1999), some have been produced in captivity (Moe 1976).

The aims of the present study were to analyze the mitochondrial DNA genetic diversity of *P. paru* and *P. arcuatus* and to determine whether there is geographic structuring of this diversity among the populations of each species collected at three sites along the coast of Brazil.

Materials and Methods

Thirty specimens of *P. paru* and *P. arcuatus* were analyzed in the present study. All specimens were collected on the coast of the states of Ceará, Pernambuco and Alagoas (Fig. 1). Thirteen were collected from a single site in Ceará (seven *P. paru* and six *P. arcuatus*). Among these *P. paru* specimens, there were two individuals (belonging to Haplotype Hp1) with a different color pattern (more grayish).

Fourteen of the specimens (12 *P. paru* and two *P. arcuatus*) were acquired from the Itamaracá Island Fishing Colony in the state of Pernambuco (7°45’17.80’’S–

34°49’26.46’’W). The remaining three specimens were *P. paru* from the state of Alagoas. The specimens from Alagoas and Ceará were donated by the proprietors of the aquarium trade companies D’água and H&K Ornamental Fishes, respectively, who offered no information on the coordinates of the collection sites (Fig. 1).

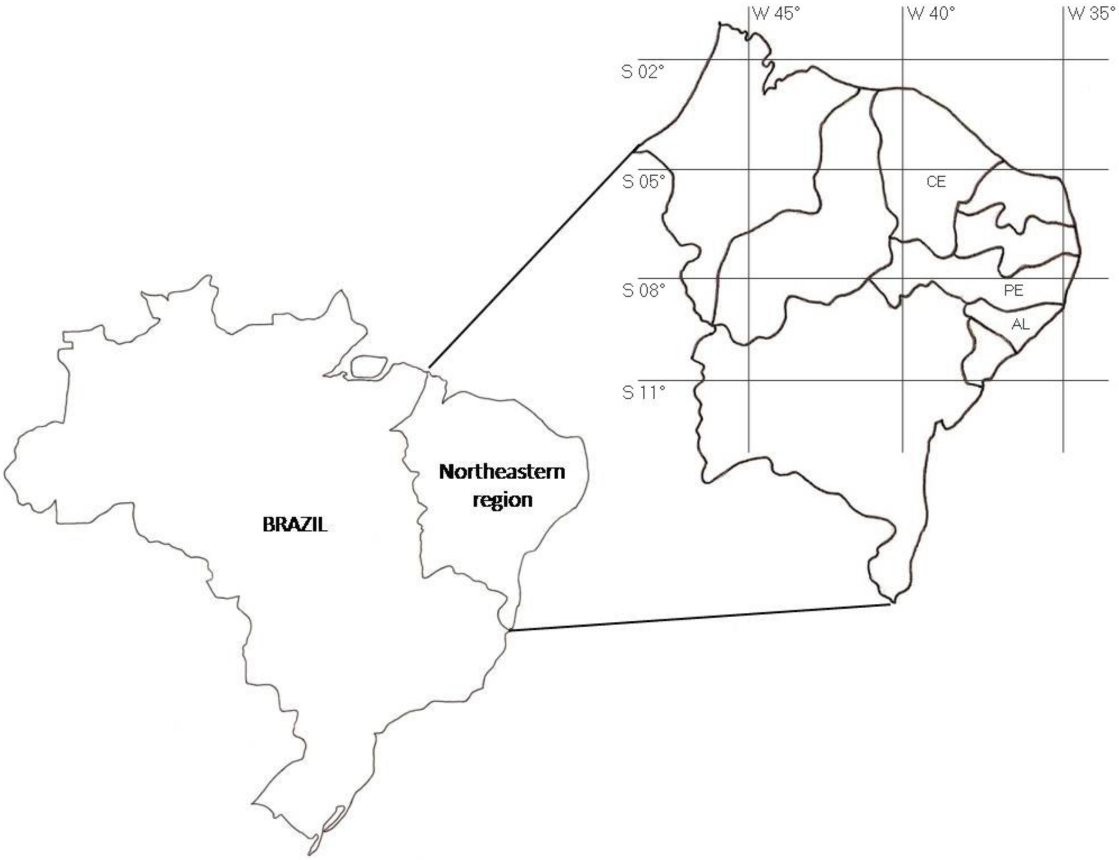

Figure 1: Location of sampled area. CE - Ceará state, PE - Pernambuco state and AL - Alagoas state.

Muscle and liver samples from each individual were stored in liquid nitrogen at the Laboratory of Applied Genetics of the Fishery Department of the Universidade Federal Rural de Pernambuco (UFRPE). The DNA extraction procedure followed the standard protocol described by Sambrook et al. (1989). Besides the collected specimens, sequences from the

GenBank were used for the specimens of *P. arcuatus* (AY530868 from the Caribbean) and *P. paru* (AY530852 from Belize) (Bellwood et al 2004).

A region of the 16S rDNA was amplified with the primers described by Simon et al. (1994): LR-J-12887 5`-CCG GTC TGA ACT CAG ATC ACG T-3` and LR-N-13398 5` CGC CTG TTT ACC AAA AAC AT 3`. PCR reactions were carried out as described in Bellwood et al. (2004). PCR products were purified using the GFX PCR DNA & Gel Band Purification Kit (GE-Healthcare) and sequenced in both directions using the DYEnamic ET Terminator Sequencing kit (GE-Healthcare) in an ABI-377 automated sequencer.

For the phylogenetic analyses, additional 16S rDNA sequences from four species of the genus *Pomacanthus* were obtained from the GenBank: *P. asfur* (AY530854), *P. semicirculatus* (AY530844), *P. sexstriatus* (AY530858) and *P. zonipectus* (AY530874). Sequences from *Chaetodontoplus duboulayi* (AY530846) and *Centropyge flavissimus* (AY530841) were used as outgroups. Nucleotide alignment was carried out by eye. A distance tree was constructed by neighbor-joining, with Kimura 2-parameter distance estimates, using the Mega 4.0 program (Tamura et al 2007). Parsimony topology was obtained using PAUP 4.0 (Swofford 1998). The bootstrap test (1000 replicates) was used in both analyses to evaluate node robustness. Network analysis was carried out for *P. paru* and *P. arcuatus* haplotypes, using the TCS software Phylogenetic Network Estimation Using Statistical Parsimony (Clement et al 2000). The choice of the ancestral haplotype was based on the central position in the network analysis as well as on frequency (Crandall and Templeton 1996). Haplotype diversity, nucleotide diversity indexes (Nei 1987), and Fu's Fs test of selective neutrlity were estimated and carried out with the Arlequin 3.01 program (Excoffier et al 2005).

Results

Six mitochondrial DNA haplotypes were found for *P. paru* (Haplotype Diversity, h= 0.67; Nucleotide Diversity, π = 0.0018) and a single haplotype was found for *P. arcuatus* (Table 1). Neighbor-joining and parsimony topologies (tree length = 154 steps; Consistency Index = 0.81) indicate that *P paru* haplotypes are monophyletic and *P. paru* and *P. arcuatus* are sister species. These topologies had a similar arrangement (Fig. 2), revealing *Pomacanthus* species to be in a monophyletic group and *P zonipectus* grouped with *P paru* and *P arcuatus*.

Table 1: Variable sites found in haplotypes of *P. paru* (Hp 1-6) and *P. acuatus* (Ha 1) and number of individuals sharing a same haplotype per locality. Sequences from the Belize specimen belonging to Hp 4 haplotype and the specimen from *P. arcuatus* from the Caribbean were obtained from GenBank.

Haplotype	**Variable position**	**Specimens / Locality**			

	`111111222233344`
	`2016667366826634`
	`6901679669235766`
		Ceará (CE)	Pernambuco (PE)	Alagoas (AL)	Caribbean

P. paru					
Hp 1 (11)	`CTACGTA-GACAT-GC`	4	5	2	
Hp 2 (1)	`..T....-.....-..`		1		
Hp 3 (1)	`.......-....C-..`			1	
Hp 4 (1)	`.......-.....T..`				1
Hp 5 (8)	`.......-.G...-..`	2	6		
Hp 6 (1)	`T......-.G...-..`	1			
P. arcuatus					
Ha 1 (9)	`.-.TTCGAT.GT.-AT`	6	2		1

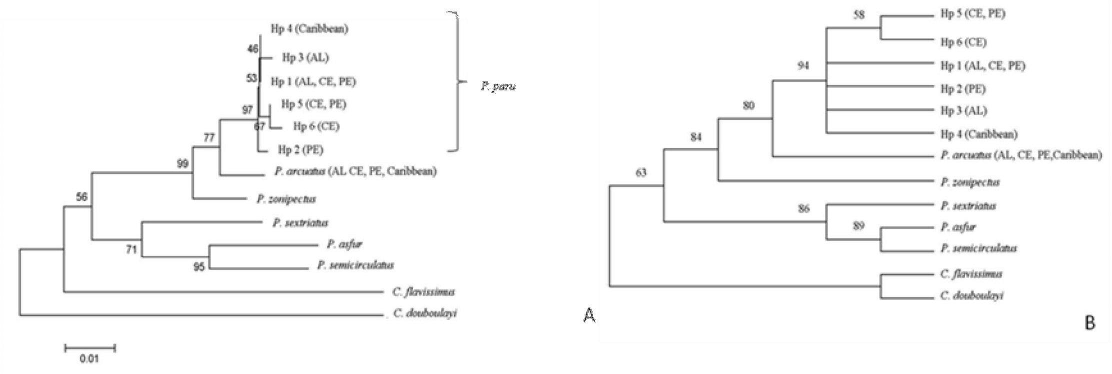

Figure 2: Neighbor-Joining (A) and Parsimony (B) topologies based on 16S rDNA mitochondrial sequences for the haplotypes of *Pomacanthus paru* and *Pomacanthus arcuatus*, and other *Pomacanthus* species. *C. flavissimus* - *Centropyge flavissimus* and *C. dubolayi* - *Chaetodontoplus duboulayi* were used as outgroups. Localities where the haplotypes were found are indicated between parenthesis: AL – Alagoas state, Brazil; CE – Ceará state, Brazil; PE – Pernambuco state, Brazil; and Caribbean. DC indicates specimens of *P. paru* with grayish coloration.

Concerning the six *P. paru* haplotypes, two had a greater frequency (Hp1 and Hp5, Table 1), with Hp1 present at all sample sites (Ceará, Pernambuco, and Alagoas). The network analysis (Fig. 3) suggests that Hp1 is the ancestral haplotype. The single haplotype of *P. arcuatus* (Ha1) was shared by individuals captured at two sites along the northeastern coast of Brazil (Pernambuco and Ceará) and is the same haplotype found in a Caribbean specimen.

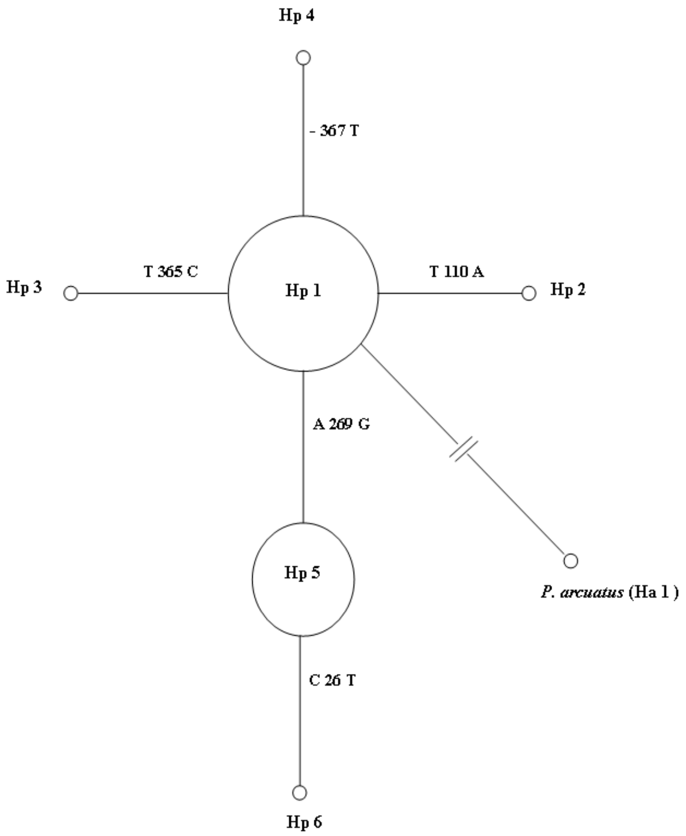

Figure 3: Parsimony Network of the mitochondrial haplotypes from *Pomacanthus paru* and *P.arcuatus*. The circles represent the haplotypes and the diameter is proportional to the number of specimens carrying each haplotype, except for *P. arcuatus* (Ha 1). Nucleotide substitutions between haplotypes were indicated.

The Fu's Fs test of selective neutrality for *P. paru* was significant marginally: Fs test = -2.35 and P = 0.022 (Fs is significant when P < 0.02) (Fu, 1997).

Discussion

Phylogeny

The molecular results obtained here indicate that *P. arcuatus* is a well-defined group, with only one haplotype identified, whereas the *P. paru* individuals form an independent clade with at least six haplotypes for the tropical southwestern Atlantic. The negative Fs value and the star-like network indicate a demographic expansion for *P. paru* populations. This hypothesis can also be extended to *P. arcuatus* with only one haplotype.

Genetic structure of species

In the tropical southwestern Atlantic the *Pomacanthus* species complex shows strong genetic heterogeneity in the populations belonging to oceanic islands, presently identified as *P. paru*, and genetic homogeneity in coastal populations classified as *P. arcuatus*. Coastal populations of *Pomacanthus* thus appear to be genetically less diverse than offshore populations of this species, paralleling results recently obtained for dolphins in the Gulf of California (Segura et al 2006). These fish species may be the result of range shifts and secondary contact after allopatric speciation, or may be indicative of parapatric ecological speciation. On the whole, *P. paru* appears to have undergone some genetic differentiation into distinct haplotypes, although the occurrence of a same haplotype in different geographical localities indicates an incomplete differentiation into distinct populations.

In a single species, the number of haplotypes may vary considerably. The calculation proposed by Nei (1987) for haplotype diversity (*h*) demonstrates that the greater it is, the greater the selective pressure on a given population will be and the more it will grow (Bowen et al., 2006). These authors consider the haplotype diversity among species of the genus *Centropyge* to be high (h = 0.991-1.0) (14 in *C. argi*, 15 in *C. resplendens* and 17 in *C. aurantonotus*). As the authors analyzed a region of mt-DNA (d-loop) that is more variable than that analyzed in the present study (16S rDNA), the value obtained for *P. paru* (h = 0.6719) could be higher if the d-loop were the molecular segment analyzed.

The present paper recognizes 6 previously unidentified haplotypes within the species *P. paru*. These evolutionary units are presently suggested by genetic divergence in the mitochondrial DNA (shifts in allele frequencies). Independent lines of research are needed in order to determine if some of these haplotypes may indicate population and species level separations (for example, constant color pattern differences, morphological differences, or reproductive isolation data**).** Our data are still inconclusive in this respect. Genetic data from samples collected along the entire ranges of the species are still needed in order to establish such ecological and geographical patterns more precisely. The identification of discrete genetic lineages within a widely distributed species is often followed by the discovery of previously unobserved morphological differentiation (genealogical concordance, and subsequent recognition of the lineages as valid species (Rocha et al 2007). Our data increase our awareness of the net biodiversity in the marine environment of the tropical southwestern Atlantic, although we have so far been unable to associate color differences with population genetic differences within *P. paru*.

The genetic pattern observed in *P. paru* may not correspond to the species evolutionary history, but merely to the history of the mitochondria (Ballard and Whittock 2004). How differentiated conspecific lineages should be before they are elevated to species level is an unresolved question, and taxonomic decisions should thus be based on the congruence of independent data sets (e.g., including sequence data from nuclear genomes, color and morphological differences, data from ecological partitions and geographical separations), rather than on absolute levels of genetic divergence.

Color Patterns

Mutations in genes that control morphological features, such as color, are commonly used to differentiate populations of marine animals (Knowlton and Mills 1992; Losos et al 1998, Endler et al 2005). The two individuals of *P. paru* sampled along the littoral of the state of Ceará with a distinct more grayish color pattern represent only a small fraction of our Hp1 haplotype. This could indicate that (1) there is no correlation between haplotype and color pattern, or (2) that there is further hierarchical structure undetected with molecular markers within *P. paru*.

A number of studies have shown that many fish populations have distinct color patterns, but these patterns are not associated with mt-DNA haplotypes (e.g., Rocha et al 2005; 2007; Schultz et al 2007). As far as our data indicate, this seems to be the case also for *Pomacanthus*. According to Rocha et al. (2007), there are two alternative explanations for this: 1) disruptive natural selection for color pattern (McCartney et al 2003; Ramon et al 2003); and 2) hybridization among color morphotypes (Puebla et al 2007). Although many species of reef fish are described based on their color patterns, morphological and genetic studies are essential for their diagnosis (Rocha 2004; Lima et al 2005).

Distribution and Ecology

Although the geographic distribution of these species coincides, *P. arcuatus* is recorded at greater latitudes in the northern hemisphere (New York), whereas *P. paru* (from Florida) reaches greater latitudes in the southern hemisphere. In Brazil, the latter species occurs as far as the state of Santa Catarina (26°58’01.67’’S and 49°22’23.45’’W), whereas *P. arcuatus* only occurs as far south as the state of Espírito Santo (19°11’22.84’’S and 40°18’33.50’’W) (Menezes et al 2003). *Pomacanthus paru* is the only species in this genus to occur in Brazilian oceanic islands, such as the Rocas atoll (Rosa and Moura 1997) and St. Peter and St. Paul’s Rocks (Feitoza et al 2003). The two species differ in abundance. *P. paru* is more common along the Brazilian coast (Carvalho Filho 1999, Ferreira et al 2004) and less common in the Caribbean (Smith 1997) in comparison to *P. arcuatus*. This observation was confirmed by Hourigan et al. (1989), who carried out a transect census in the Caribbean (St. Croix, US Virgin Islands) and obtained a greater average density for *P. arcuatus*

(0.56/1000m2) in comparison to *P. paru* (0.31/1000m2). Based on the fact that *P. arcuatus* is more frequent in the Caribbean, it is possible that *P. paru* is more apt to occupy more recent habitats, such as those found along the Brazilian coast.

For a definitive picture of management units within *P. paru*, it will be necessary to survey the entire geographical and ecological range of the species, in order to fully understand the phylogeography, or geographical distribution of genetic lineages. For cases in which sibling species complexes occur in sympatry and show no clear differences in ecological specialization, Gittenberger (1991) has used the term nonadaptive radiation.

The partial overlap between the geographical ranges of *P. acuatus* and *P. paru*, and also among the haplotypes of the latter species, indicates that conventional allopatric speciation by biogeographical barriers (e.g., Bernardi et al 2003) is not the only explanation for the speciation of tropical reef fishes. Parapatric ecological speciation by habitat partitioning at a local scale represents a mechanism that may explain the long-standing enigma of high biodiversity in coral reef faunas (Rocha et al 2005).

Conclusions

Our data support the phylogenetic proximity between *Pomacanthus paru* and *P. arcuatus* and corroborate morphological, ethological (Pyle and Randall 1994), enzymatic (Chung and Woo 1998) and chromosomal (Galetti et al 2006) patterns. The molecular phylogeographical results of the present study confirm the taxonomic status of these sister species and suggest that, although they are sympatric (frequently syntopic), they have different evolutionary histories.

The considerations presented here represent a first step toward understanding the population structures of the sister species *Pomacanthus paru* and *P. arcuatus*, which have economic value as ornamental fish. Complementary studies on larval settlement, physiological characteristics and anthropogenic actions directed at these populations are needed for a greater understanding of the dynamics of these species. The knowledge obtained herein on genetic distinctions into distinct haplotypes of *P. paru* has important conservation implications, considering that population diversity of this commercial fish may be larger than previously thought.

Although our paper deals primarily with the intraspecific genetic diversity of a commercial group of reef fish, stretches of mtDNA have nevertheless been shown to have tree-like histories within a species, and the branching structures of these gene genealogies may bear considerable analogy to phylogenetic trees summarizing historical relationships among reproductively isolated taxa and higher taxa, albeit on a different time scale (Avise 2009).

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Capítulo 4 - **Um método novo e rápido para avaliar a sustentabilidade do bycatch de peixes ornamentais dos recifes de corais.**

Chapter 4*† - **A rapid new method for assessing sustainability of ornamental fish bycatch from coral reefs.**

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A rapid new method for assessing sustainability of ornamental fish bycatch from coral reefs.

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Abstract

The ornamental fish trade has a considerable potential for damaging reef environments. Nearly 120 species are currently harvested in the Brazilian trade and little attention has been paid to the effects of the accidental catch of these species. Records of ornamental fish bycatch and the susceptibility and resilience of species from the major Brazilian families in the aquarium trade were examined to test a method and determine the sustainability of these catches. Each species was assessed based on two groups of criteria: (1) relative vulnerability to capture; and (2) population resilience regarding mortality due to fishing efforts. Each species was also ranked (1-3) based on individual criteria regarding ecology, biology, catchability, numbers of individuals exported by aquarium companies and mortality index. These criteria were then weighted and each species rank in the two groups of criteria was plotted. Nineteen species from 10 families were recorded. The species least likely to have sustainable catch rates were *Holacanthus tricolor*, *H. ciliaris*, *Chaetodon striatus* and *Pomacanthus paru*. This approach is a useful alternative for management efforts and the assessment of catch sustainability in newly-developed multi-species fisheries with limited available data, which occur in areas of high species richness such as reefs.

Additional keywords: aquarium, trap, fishery, susceptibility, resilience, Pomacanthidae, Chaetodontidae.

Introduction

Species classified as bycatch (Eayrs 2007) are caught in fisheries that target other groups and are then either used or discarded. It is estimated that 7.3 million tonnes of fish were discarded globally in commercial fisheries in the period of 1995-2005 (Kelleher 2005). This is equivalent to 8% of worldwide marine fish production (Eayrs 2007). The global effect of fisheries on bycatch species is poorly known, although some studies have documented ecological changes on the species level (Casey and Myers 1998; Pope *et al.* 2000). Even less attention has been paid to accidental catches affecting reef fish, including ornamental species.

Beauty and perfect patterns on ornamental reef fish are requirements imposed by the international aquarium trade (Araújo and Albuquerque Filho 2005), which is one of the world's largest industries. The import value worldwide of marine fish and invertebrates is estimated to be between US$ 24 and 40 million annually (Wood 2001a). Nearly 120 species are currently harvested in the Brazilian aquarium trade (Monteiro Neto *et al.* 2003). Most belong to the Pomacanthidade family (Araújo and Albuquerque Filho 2005), while the Chaetodontidae are among the most exported (Monteiro Neto *et al.* 2003; Nottingham *et al.* 2000). Although other fisheries that use traps have an effect on ornamental marine fish stocks (through by-catch), these stocks have only been managed through a Federal Act (56/04, established in 2004 by the Brazilian environmental protection agency – Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA) that monitors ornamental fish trade through export quotas. The purpose of this Federal Act is to administer this activity through the establishment of export quotas.

Traps are the predominant gear employed to capture reef fish in the Caribbean (Swingle *et al.* 1970; Garrison *et al.* 1998) as well as other places in the world (e.g. Arabian Gulf, Seychelles, Papua New Guinea) (Dalzell 1996) and are responsible for the decline of many reef species, which are fishery targets (Wolff *et al.* 1999). Among the organisms caught by these devices, the most frequently caught ornamental fish belong to the following genera: *Acanthurus*, *Chaetodon*, *Bodianus*, *Holacanthus* and *Pomacanthus* (Garrisson *et al.* 1994; Wolff *et al.* 1999). In Brazil, trap fisheries targeting reef fish have been on the rise in the last ten years (Ribeiro 2004). The target species of these fisheries belong to the genera *Acanthurus*, *Cephalopholis*, *Lutjanus*, *Pseudupeneus* and *Sparisoma* (Ribeiro 2004). Because by-catch, including ornamental species, is returned to the sea and not recorded, there are no data or regulations available about the actual catch of ornamental species.

Due to the increasing pressure to manage the impact of fisheries on non-target species and the uncertainty of how this is to be done, Stobutzki *et al.* (2001) has developed a broad method using qualitative measures of the biological and ecological characteristics of species to rank vulnerability to a known threat. These authors examine the impact of trawling on the catch sustainability of teleost bycatch species, identifying the species with the least likelihood of catch sustainability in bycatch. Such species should then be the focus of research and management. The authors have recorded at least 411 species from 99 families as bycatch, identifying 15 species with the least likelihood of catch sustainability as well as the 13 with the most likelihood of catch sustainability. This approach was designed to overcome problems such as the high diversity of bycatch in tropical fisheries and the lack of information on individual species. The method is versatile and can also be applied to other fisheries, although the criteria employed may vary.

The present study describes a rapid, innovative method, based on the method developed by Stobutzki *et al.* (2001), for evaluating the impact of trap fisheries on ornamental species using biological data and the portion of the population caught. The intention is to answer the following two questions. (1) Is the method appropriate for ornamental reef fish? (2) Is the capture of ornamental reef fish as by-catch sustainable? The present study records the occurrence of ornamental fish caught as by-catch and assesses the susceptibility and resilience of fish from the Pomacanthidae and Chaetodontidae families.

Materials and Methods

Sampling

The fish recorded here were acquired from the fishing colony of Itamaracá island located on Pilar beach (7°45’17.80’’ S – 34°49’26.46’’W) (Itamaracá, state of Pernambuco, Brazil – Fig. 1) and were caught as bycatch while trap fishing for the spotted goatfish, *Pseudupeneus maculatus* and parrotfish, *Sparisoma* spp. This kind of fishing occurs throughout the year and is carried out on alternating days. The boats leave Pilar beach at midnight and arrive at Tambaba beach, 46 km away, (7°22’2.93’’S – 34°47’48.51’’W) (state of Paraíba) in the early morning. Four boats are employed in this fishery. Each has a fishing effort of 15 to 20 traps and trap mesh varying between 1 and 7 cm. The traps are deployed unbaited next to reefs and hauled 6 hours later. Occasionally, depending on factors such as wind and currents, some traps are left for longer periods and only hauled after 48 hours. The fish are stored for approximately 7 hours in 100-L containers. For the present study, weekly samplings were performed from April to June 2006. The fishermen were asked to bring back all of specimens with ornamental characteristics, which were then bought. In the case of *H. ciliaris* and *P. paru*, this procedure was carried out over a longer period (March 2006 to February 2007), as these two species were the focus of another study.

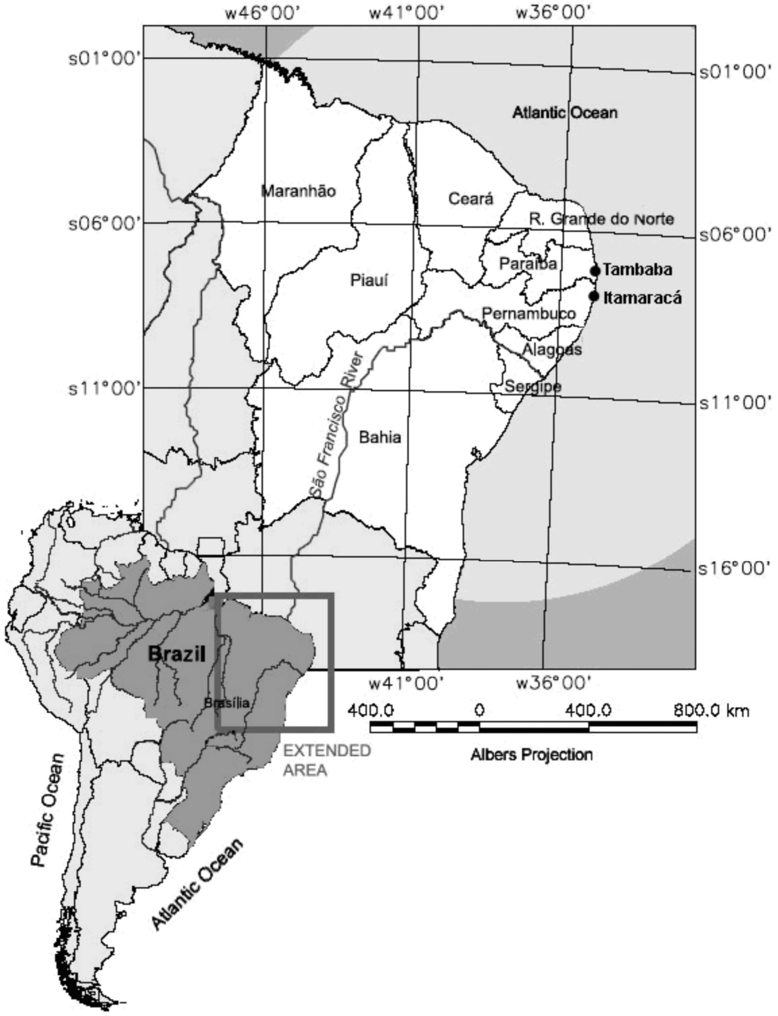

Figure 1: Location of sampled area.

The sampled fish were placed in polystyrene chests with ice and taken to the Nekton and Aquaculture Laboratory of the Oceanography Department of the Universidade Federal de Pernambuco, where they were identified, measured and photographed.

Analysis of catch sustainability of ornamental reef fish bycatch

Only the most abundant species were analysed, i.e., species from the Pomacanthidae and Chaetodontidae families. Rare species for Brazilian northeastern coast, such as *C. ocellatus* and *P. arcuatus* (Thresher 1980; Carvalho Filho 1999) were not surveyed due to the small sample size. Information on the biological characteristics of the selected species was taken from specific publications, such as Robins and Ray (1986); Thresher (1980, 1984); Allen (1998), Hourigan *et al.* (1989) and Froese and Pauly (2007).

The method proposed to access the sustainability of selected bycatch species was adapted from Stobutzki *et al.* (2001) and applied based on two scored sets of criteria: (1) relative vulnerability to capture by traps and mortality due to trap fishery; and (2) the recovery capacity of a species after the population is depleted. The criteria were originally developed during a study on the sustainability of all North Australian Prawn Fishery (NPF) prawn-trawl vertebrate bycatch (Stobutzki *et al.* 2000). These criteria were modified and assigned relative importance weights by the NPF Assessment Group (Milton, 2001). Values ranging 1 to 3 were attributed to each sustainability indicator, for which 1 denotes the highest value for the susceptibility criteria and the lowest for resilience. Depending on the criterion, these values were based on either categorical or continuous data. When no information was available to assign the divisions between values, the range of the data was divided into thirds in order to create categories (Table 1). The rank matrix constructed from these values for each set of criteria are plotted as axes (resilience *versus* susceptibility) on a graph indicating the species with the greatest catch sustainability. This graph is recommended only if the different species have different values on each axis. According to Stobutzki *et al.* (2001), when species-specific information is unavailable, a species is given the same value as other species within the same family. With criteria for which family members are not similar or for which no family information is available, the rank of 1 is given as a precautionary measure.

The criteria adopted to analyse species susceptibility (axis 1) to capture and possible mortality due to fishery efforts were adapted from Stobutzki *et al*. (2001) and are summarised as follows: (1.1) water column position – as traps are deployed on the substrate (Mahon and Hunte, 2001; Garrisson *et al.* 2004), benthic and demersal species are more susceptible to capture than the pelagic species; (1.2) preferred habitat – species are ranked according to their preference for rocky habitats. This criterion addresses the likelihood that a species habitat overlaps the area in which trap fishery occurs. Coral reef habitats have been historically targeted by trap fisheries (Wolff *et al.* 1999). Thus, species that live near shore and depend on a specific habitat, such as coral reefs, are highly vulnerable to a possible risk of extinction (Dye *et al.* 1994; Roberts and Hawkins, 1999); (1.3) day/night catchability – as trap fishery is a daytime operation, species with diurnal habits are more susceptible to capture by traps; (1.4) diet – addressing whether eating habits attract species to trap fishery areas. Thus, species that generally feed on benthic or demersal organisms found on reefs are more vulnerable to capture. Invertebrate feeders (mobile and sessile) are the dominant trophic guild in traps deployed in coral reef habitats (Garrison *et al.* 2004); (1.5) depth range – this trap fishery occurs in areas between 30 and 40 m. According to Wolff *et al.* (1999), trap fisheries in adjacent coral reef and gorgonian habitats range from 3 to 38 m. Overlap between the depth range of the trap fishery area and that of the preferred habitat will affect the vulnerability of a species to capture more than that of others that live in either deeper or shallower areas. Fish that have a narrow vertical depth range are highly vulnerable to extinction (Dye *et al.* 1994; Roberts and Hawkins, 1999) (Table 1).

Six criteria adapted from Stobutzki *et al*. (2001) were employed to analyse species resilience: (2.1) the maturity criterion – if most individuals caught are larger than their size at first maturity, the species has a greater probability of recovering from a declining population; (2.2) maximum size – species that attain large sizes tend to live longer and therefore the resilience of the population is lower than species with smaller body sizes. A slow life history (e.g. late maturity, long reproductive span and large body size) is related to low maximum population growth rates and, consequently, such species are more vulnerable and have less capacity to recover from exploitation (Hutchings 2001; Reynolds *et al.,* 2001, 2002); (2.3) removal rate – species-based quotas established by the Brazilian environmental protection agency (IBAMA) to ensure that harvests are maintained at a sustainable level. The ornamental species fishery is highly selective and certain species such as chaetodonthids and pomacanthids are preferred targets. Among those, the species which are rare may be vulnerable to over-exploitation (Wood 2001b); (2.4) reproductive strategy – species capable of dispersion over long distances are less vulnerable to extinction (Dye *et al.* 1994; Roberts and Hawkins, 1999). Thus, the resilience of R-strategist species is generally higher than that of K-strategist species; (2.5) hermaphroditism – hermaphroditic species, especially protandric species, may be at great risk of extinction, as fisheries select individuals of certain sizes, which has a negative effect on reproduction (the fishing effort is directed at only one sex) (Dye *et. al.* 1994; Roberts and Hawkins, 1999; Hawkins and Roberts, 2003); (2.6) mortality index – this index was proposed by Sparre and Venema (1992) and Stobutzki *et al.* (2001). This index can be derived from the length frequency of a species and von Bertalanffy growth parameters. However, such parameters are not available for most bycatch species. Thus, the index can be calculated as follows:

Mortality index (Z): (L max – L mean)/ (L mean – L min)

in which L max is the maximum length recorded in the literature; L mean is the mean length of total number of individuals caught; L min is the minimum length of the individuals in the sample. In the present study, the mortality index range was calculated (0.13 – 0.74) and divided into thirds for the division between ranks (Table 1).

Table 1: The criteria used to assess the susceptibility of bycatch species to capture and mortality due to traps and their recovery. For each criterion the weighting score and definition of the three ranks is given.

Criteria	Weighting score	**1**	**2**	**3**
Susceptibility				
Water column position	3	Demersal or benthic	Benthopelagic	Pelagic
Preferred habitat	3	Rocky habitats with gravel and sand	Habitats with soft or muddy bottoms	No fishing habitats
Day/night catchability	2	Higher catch rate at day	No difference between day and night	Higher catch rate at night
Diet	3	Feed on demersal or bent. organisms	Feed on benthopelagic organisms	Feed on pelagic organisms
Depth range	1	Less than 40m	-	Deeper than 40m
Resilience				
Maturity	3	Mature individuals < 50%		Mature individuals > 50%
Maximum size	3	Max. size ≥ 50 cm	16 cm < Max. size < 50 cm	Maximum size ≤ 16 cm
Removal rate	3	Catch ≥ 3500 inds/comp/yr	1000 < catch < 3500 inds/comp/yr	Catch ≤ 1000 inds/comp/yr
Reproductive strategy	2	Bear live young or brood young	Guard eggs and/or young	Broadcast spawners
Hermaphroditism	1	Protandric hermaphrodites	Protoginic hermaphrodites	Dioecious
Mortality index	1	Mortality index ≥ 3.19	0.96 < mortality index < 3.19	Mortality index ≤ 0.96

Caption: bent – benthic; Max – Maximum; inds/comp/yr – individuals/company/year

Analysis of criteria

The overall ranks of susceptibility or resilience were calculated according to the equation below:

$$Si = \sum_{j=1}^{n} wj\, Ri \;/ \sum_{j=1}^{n} wj$$

in which Si is the total susceptibility or resilience rank for species i; wj is the weight for criterion j; Ri is the rank of species i for criterion j; and n is the number of criteria for each axis.

Statistical analysis

Data normality was evaluated using the Kolmogorov-Smirnov test (BioEstat 5.0 software – Instituto de Desenvolvimento Sustentável Mamirauá). The parametric Z test (BioEstat 5.0 software) was used to determine the probability of a captured individual being smaller than size at first maturity and the probability of a captured individual having bred at least once. Correspondence analysis (Multivariate Statistical Package – MVSP 3.1 software – Kovach Computing Services) was used to determine and describe the correspondence between species and criteria on a same graph. This multivariate analysis was used only for criteria related to resilience, as values regarding susceptibility did not vary among species. Since the variance of the reproductive strategy criterion was equal to zero, this criterion was not applied in the present analysis.

Results

Among the fish caught, 19 were recorded as bycatch species and belong to 14 genera and 10 families (Table 2). Of these, 100% come from reef environments and are used in the aquarium trade and only one species (*Equetus lanceolatus*) was not authorised for export. A total of 430 individuals were sampled: 147 *H. ciliaris*, 46 *H. tricolor*, 200 *P. paru* and 37 *C. striatus*. Length frequency distribution by species (Fig. 2) revealed a broad length range among the individuals sampled.

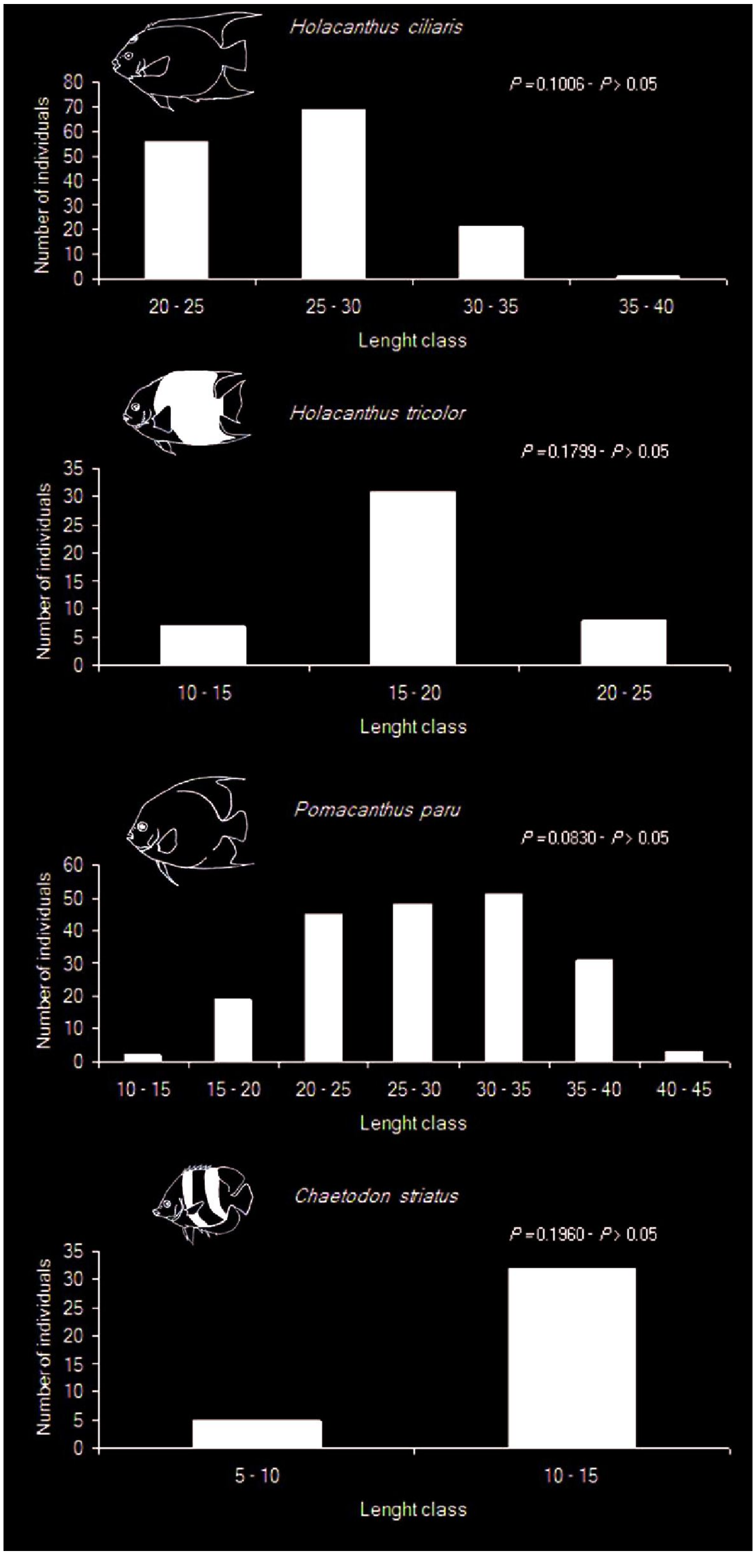

Figure 2: Length distributions for the four analyzed species. P values refers to normality.

Catch sustainability of ornamental fish bycatch...

Table 2: Species registered as bycatch, with the respective common names.

Family	**Species**	**Common name**	**n**
Sygnathidae	*Hippocampus reidi* Ginsburg, 1933	Longsnout seahorse	2
Synodontidae	*Synodus foetens* (Linnaeus, 1766)	Inshore lizardfish	2
Aulostomidae	*Aulostomus maculatus* Wheeler, 1955	Trumpetfish	1
Sciaenidae	*Equetus lanceolatus* (Linnaeus, 1758)	Jack-knife fish	2
Sciaenidae	*Pareques acuminatus* (Bloch & Schneider, 1801)	Highhat	1
Chaetodontidae	*Chaetodon ocellatus* Bloch 1787	Spotfin butterflyfish	2
Chaetodontidae	*Chaetodon striatus* Linnaeus, 1758	Banded butterflyfish	37
Pomacanthidae	*Holacanthus ciliaris* (Linnaeus, 1758)	Queen angelfish	147
Pomacanthidae	*Holacanthus tricolor* (Bloch, 1795)	Rock beauty	46
Pomacanthidae	*Pomacanthus arcuatus* (Linnaeus, 1758)	Gray angelfish	3
Pomacanthidae	*Pomacanthus paru* (Bloch, 1787)	French angelfish	200
Labridae	*Bodianus rufus* (Linnaeus, 1758)	Spanish hogfish	23
Labridae	*Bodianus pulchellus* (Poey, 1860)	Spotfin hogfish	3
Labridae	*Halichoeres dimidiatus* (Agassiz, 1831)	Brazilian yellowcheek wrasse	31
Labridae	*Halichoeres brasiliensis* (Bloch, 1791)	Brazilian wrasse	10
Monacanthidae	*Cantherhines macrocerus* (Hollard, 1855)	Whitespotted filefish	1
Monacanthidae	*Stephanolepis hispidus* (Linnaeus, 1766)	Planehead filefish	1
Ostracidae	*Lactophrys trigonus* (Linnaeus, 1758)	Buffalo trunkfish	9
Tetraodontidae	*Canthigaster figueiredoi* Moura & Castro (2002)	Southern Atlantic sharpnose-puffer	1

All information on susceptibility criteria is species-specific and the four species evaluated in the sustainability analysis obtained the same value (1) for this criterion. Rank resilience ranged from 1.92 to 2.54; *H. tricolor* and *P. paru* obtained the smallest and the largest values, respectively (Table 3). Based on these results, it can be affirmed that *H. tricolor* has the least catch sustainability, followed by *H. ciliaris* and *C. striatus*. *P. paru* is the most capable of recovering from a population decline.

Mean and smallest lengths for *H. tricolor* and *C. striatus* individuals were under size at first maturity. There was a 71% probability of *H. tricolor* individuals and a 92% probability of *C. striatus* being caught at sizes smaller than first maturity. Contrarily, the probability of captured individuals being larger than maturity size was 76% for *H. ciliaris* and 70% for *P. paru*. *H. ciliaris* had the highest mortality index (Z=3.19) and *P. paru* had the lowest (Z=0.96) (Table 4).

Table 3: The ranking of the species on the criteria on the resilience axis.

	Resilience						
Species/Weight	**3**	**3**	**3**	**2**	**1**	**1**	**Rank**
	Maturity	Maximum size	Removal rate	Reproductive strategy	Hermaphroditism	Mortality index	
Holacanthus ciliaris	3	2	1	3	2	1	2.08
Holacanthus tricolor	1	2	2	3	2	2	1.92
Pomacanthus paru	3	2	2	3	3	3	2.54
Chaetodon striatus	1	3	3	3	3	2	2.46

Table 4: The length at first capture, length at maturity, probability of individuals caught are below of the length at maturity, probability of breeding, mean and maximum length (Max. length) of the species and the mortality index (Z) of each.

Species	n	L' (cm)	Lm (cm)	Prob. L < Lm (cm)	Prob. of breeding	L mean ± S. E (cm)	Max. Length (cm)	Z
H. ciliaris	147	20.88	24.3	p = 0.24	p = 0.76	26.58 ± 0.2672	45 (Allen, 1998)	3.19
H. tricolor	46	11.32	19	p = 0.71	p = 0.29	17.61 ± 0.3646	35 (Allen, 1998)	2.68
P. paru	200	13.44	25	p = 0.30	p = 0.70	28.30 ± 0.4510	41 (Cervigón, 1993)	0.96
C. striatus	37	9.09	13.2	p = 0.92	p = 0.07	11.26 ± 0.2257	16 (Lieske and Myers, 1994)	2.17

Caption: n – number of individuals, L' – length at first capture, Lm – length at maturity, Prob – probability, Max. Length – maximum length, Z – mortality index.

The correspondence analysis for resilience revealed that *H. tricolor* and *C. striatus* are linked to the removal index. *H. ciliaris* and *P. paru* are associated through maturity criteria. *Chaetodon striatus* and *P. paru* are grouped with maximum size and mortality index criteria, respectively. Negative relationships were determined for *H. ciliaris* and the removal rate and mortality index; *H. tricolor* and *C. striatus* with maturity criteria; and *P. paru* with maximum size. The percentage of the cumulative variation for the two axes was 99.53% (Fig. 3).

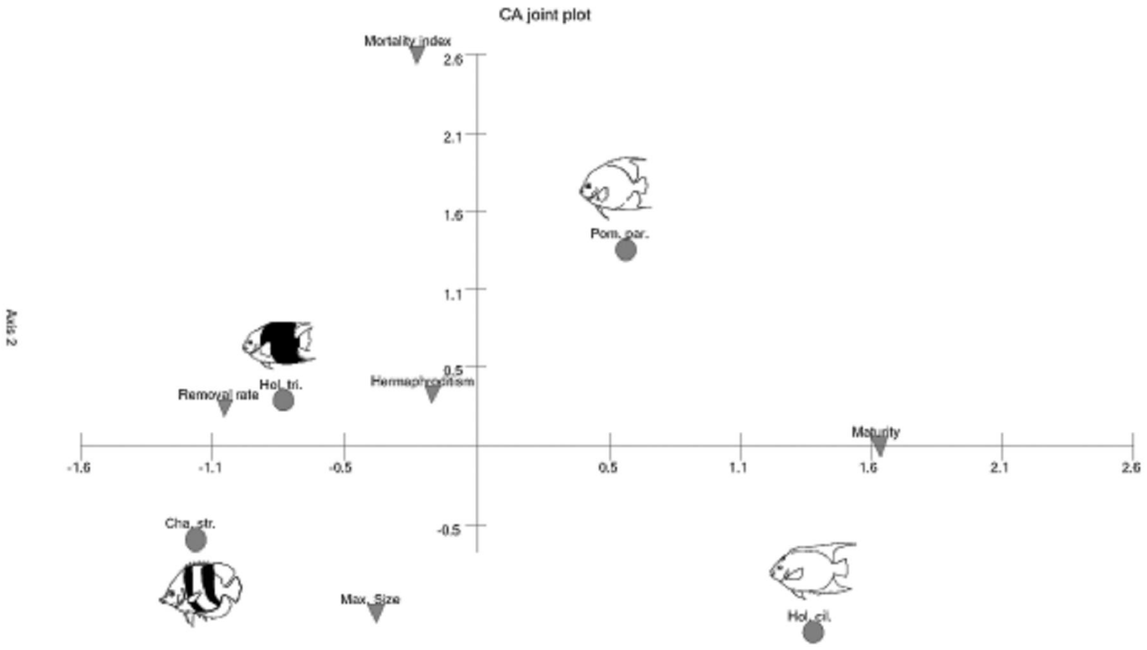

Figure 3: Correspondence analysis showing the relationship between species and criteria used to evaluate the recovery capacity.

Caption: *Hol. cil. – Holacanthus ciliaris, Hol. tri. – Holacanthus tricolor, Cha. str. – Chaetodon striatus, Pom. par. – Pomacanthus paru*, Max. Size – Maximum size.

Discussion

The present study demonstrates that trap fishery is not selective. The catching of ornamental fish occurs frequently and likely causes considerable impact. Ornamental species are already considered seriously endangered due to fishery efforts (Cheung *et al.* 2007). Individuals caught are not landed and are usually returned to the sea (many still alive, according to the fishermen). Despite this, the survival of these individuals is doubtful, as the traps are hauled quickly to surface and problems related to decompression have often been reported (Burns and Restrepo 2002).

The method applied in the present study was developed with the purpose of classifying ornamental fish species according to possible risk of extinction due to being captured as bycatch. This analysis is influenced by the length range and proportion of young and immature individuals sampled, as criteria that are directly related to specimen size are employed, such as length at first maturity and mortality index. Different mesh sizes are used in this fishery activity, which may have contributed toward the broad length range among the individuals sampled. Due to the small sample size for *H. tricolor* and *C. striatus*, the length range of the specimens caught may not be representative. Thus, for these species, the analysis must be considered only for demonstration (exploratory) purposes, even though there was a trend toward normality in the length distribution data. The present study is the first to test a method with such goals directed at trap fisheries and using criteria based on life history.

Specific biological information is optimised when well-defined criteria are applied, including characteristics that affect species sensitivity or risk of extinction (Dye *et al.* 1994; Roberts and Hawkins 1999; Stobutzki *et al.* 2001). Stobutzki *et al.* (2001) apply the value 1 in the matrix when there is no specific information on certain criterion. In the present study, all biological information was species-specific. Although the analysis presented is subjective, this may be the only one available to evaluate and monitor multi-specific fisheries (Dulvy *et al.* 2003), especially newly developed fisheries.

Susceptibility to capture by traps

Species caught by a given equipment share similar characteristics, such as habitat, diet and period of activity. Thus, reef fish are considered highly susceptible to trap fisheries. Pomacanthidae are widely distributed in depths ranging from 1 to 100 m (Allen 1998; Michael 2004; Froese and Pauly 2007), whereas Chaetodontidae occur between 3 to 55 m (Allen 1998; Robins and Ray 1986; Froese and Pauly 2007). The former feed on sponges, zoanthids, tunicates, hydroids, bryozoans and algae (Randall 1967; Allen 1998; Michael 2004). Chaetodontidae eat worms, coral polyps and mollusc eggs (Allen 1998). The diet and period of activity of these fish favour their capture, as they only feed during the day (Starck and Davis 1966) and the organisms they eat are sessile, which are generally encrusted on reefs. Dependence on a particularly susceptible habitat (e.g. coral reefs), either alone or combined with overfishing, leads to considerable captures of bycatch species and makes these species all the more vulnerable (Reynolds and Jennings 2000).

Resilience

Species with late sexual maturity are highly vulnerable to the risk of extinction and consequently have a poor recovery capability (Dye *et al*. 1994; Roberts and Hawkins 1999; Sadovy 2005). The correspondence analysis reveals the clustering of *H. ciliaris* and *P. paru* with the maturity criterion, due to the fact that more than a half of the sampled individuals had bred at least once. The opposite was found for *H. tricolor* and *C. striatus*, for which respectively 70% and 92% of individuals sampled were immature. Myers and Worm (2005) state that the risk of extinction is very high if recruits are being captured and low when fish are caught after first sexual maturity. Thus, the maturity criterion had a positive influence on the classification of *H. ciliaris* and *P. paru* in relation to resilience.

Large body size is often correlated with other vulnerability factors, such as late maturity, and substantially increases the possibility of being caught in traps (Reynolds and Jennings 2000; Reynolds *et al*. 2002, Cheung *et al*. 2007). Body size is a useful measure in evaluating species resilience due to its relationship with the intrinsic rate of increase of a population. Thus, it can be applied to populations and species for which minimal biological data are available (Denney *et al*. 2002). In the Pomacanthidae family, the genera *Holacanthus* and *Pomacanthus* have the largest lengths (Bellwood 2004). Among the analysed fish, *P. paru* was the second largest, but exhibited the greatest resilience. This can be explained by the fact that most individuals caught were fit for reproduction and the species had the lowest mortality rate.

The demand for marine ornamental fish increased in the 1980s and 1990s (Chan and Sadovy, 1998). Concerns have been raised regarding its negative effect on reef fish and coral habitats, as nearly all ornamental marine fish are taken from the wild (Andrew 1990; Wood 2001a). The potential for over-exploitation from the aquarium trade is high due to the high selectivity of this activity and the large number of fish collected (Wood 2001a). It is estimated that most commercialised ornamental fish are juveniles and less than 10 cm in standard length (Chan and Sadovy, 1998). Pomacanthidae and

Chaetodontidae are often the most important families for export in terms of number and value (Wood 2001a). In the Brazilian aquarium trade, these two families are among the most representative in species richness (Gasparini *et al.* 2005). In the state of Ceará (one of the main centres of the Brazilian ornamental fish trade), *H. ciliaris*, *P. paru*, *Hippocampus erectus*, *Pomacanthus arcuatus* and *Holacanthus tricolor* made up 50% of the total trade between 1995 and 2000 (Monteiro Neto *et al.* 2003). Among the species evaluated, *H. ciliaris* is the most harvested by the Brazilian aquarium trade (IBAMA - Federal Act 56/04). The correspondence analysis corroborates this statement, revealing a negative relationship between *H. ciliaris* and removal rate.

Regarding reproductive strategy, all species evaluated are broadcast spawners (Thresher 1980; 1984). Therefore, they are less vulnerable to extinction due to their widespread distribution and the high re-colonisation potential of depleted populations (Malakoff 1997; McKinney 1997). However, larval behaviour, spawning events and local oceanography processes can influence larval dispersion by increasing local retention (Cowen *et al.* 2006; Rocha *et al.* 2007). Hawkins *et al.* (2000) state that, despite reef fish being broadcast spawners, their geographic distribution is small; 24% are restricted to less than 800,000 km² and 9% are restricted to less than 50,000 km². Based on these statements, it is possible to infer that being a broadcast spawner does not ensure low vulnerability to extinction.

Hermaphroditic species are likely to be very sensitive to over-fishing, as individuals need to attain a certain length for the gender change to occur (Francis 1992). If fishing affects the growth of the fish such that sexual inversion does not occur, this could result in a non-reproductive population and even cause the species to disappear in intensely exploited areas (Hawkins and Roberts 2003). Among the species analysed in the present study, those from the *Holacanthus* genus are protogynous hermaphrodites (Thresher 1980; 1984; Allen 1998; Moyer *et al.* 2003). These species are heavily exploited by the aquarium trade, in which most individuals collected are small in size (Chan and Sadovy 1998) and probably belong to the same sex (i.e., female). *H. tricolor* and *H. ciliaris* revealed the lowest recovery capacity. However, according to the correspondence analysis, the hermaphroditism criterion had no influence over their classification regarding resilience. This may be explained by the absence of protandric species in the analysis, as such species are more sensitive than protogynous species (Sadovy and Shapiro1987; Hawkins and Roberts 2003).

Throughout the life cycle, individuals are exposed to different types of environmental pressure. The natural mortality rate decreases as the fish grows and becomes less vulnerable to predators. The mortality coefficient used in the present study considered data on maximum and mean catch length (Beverton and Holt 1956). *H. ciliaris* had the highest mortality index among the species analysed due to the mean catch size, which was below that found in the literature (Allen 1998). This coefficient – along with the criterion related to the removal rate – had a negative influence on the resilience of this species.

Resilience ranks were different for the four species analysed, even for those belonging to the same family. Stobutzki *et al*. (2001) state that the catch of a species is not sustainable if it has both high resilience and susceptibility. The results obtained for *C. striatus* and *P. paru* are in agree with this statement, as these species revealed considerable resilience, but were highly susceptible according to the ecological information (such as diet, preferred habitat and depth range). This suggests that catching these species is not a sustainable practice.

The method employed in the present study (susceptibility and resilience criteria) is efficient for evaluating the impact of recently-formed fisheries with few available data that occur in areas with high species richness (Dulvy *et al*. 2003, 2004), such as reef environments. However, in order to incorporate the results of this study to fishery management measures, the vulnerability analysis (VA) can be utilised as a component of a risk analysis when only qualitative data is available and researchers lack an understanding of cause/effect relationships (Walker et al. 2002; McFadden 2007).

Most fishing equipment is not selective and many fish are caught as bycatch even when there is only one target species. These species are highly affected by fishery efforts, as bycatch will continue to be fished as long as it remains viable to catch the target species. Trap fisheries targeting reef fish have recently developed in Brazil and are on the rise in the north-eastern region. Regulatory agencies often take a long time to react and severe declines in non-target species go unperceived. Thus, little is known regarding the impact of this activity and such lack of information hinders regulatory efforts. The method presented here is an appropriate, practical tool for fishery management and the assessment of catch sustainability in multi-species fisheries on which there is limited available data. It can also be employed to determine which species are at a possible risk of extinction due to bycatch. Furthermore, it is important to consider that to reduce the extinction threat posed on those species, a socio-economic vulnerability analysis is required in order to develop the appropriate institutional framework/outreach program to the fishers necessary to address this ecological challenge.

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Considerações finais

O conhecimento do ciclo de vida de espécies como *Pomacanthus paru*, que são extraídas diretamente dos recifes para o aquarismo, é fundamental para a sua preservação. Desde o ano 2000, esta espécie vem sendo classificada como a segunda mais exportada pelo Brasil, principalmente pelo Estado do Ceará. Nas últimas reuniões técnicas sobre a exploração de peixes ornamentais marinhos, promovidas pelo IBAMA, foi enfatizada a carência de pesquisas sobre a biologia e a dinâmica populacional das principais espécies exportadas (e.g. *Holacanthus ciliaris* e *Pomacanthus paru*).

As considerações aqui apresentadas sobre a diversidade genética dos peixes anjo representam um primeiro passo para se compreender as estruturas populacionais de espécies irmãs e simpátricas, como *P. paru* e *P. arcuatus,* embora com histórias evolutivas diferentes. Estudos complementares sobre assentamento larval, características fisiológicas e ações antrópicas dirigidas a essas populações são necessários para inferir sobre essa dinâmica.

As pesquisas relacionadas à biologia reprodutiva indicaram que *P. paru* é gonocorística e os dados de reprodução, idade e crescimento forneceram informações importantes sobre sua biologia populacional. Algumas hipóteses, como a preferência de habitat entre os sexos, uma maior susceptibilidade de captura das fêmeas por meio das armadilhas e a migração das fêmeas no período reprodutivo para a área de pesca, podem explicar a prevalência de fêmeas na estrutura populacional de *Pomacanthus paru*. Entretanto, mais pesquisas serão necessárias para responder esta questão.

Os resultados mostraram também que é possível obter estimativas de crescimento para espécies tropicais baseadas em métodos convencionais para determinação de idade. Os dados obtidos nos trabalhos de biologia reprodutiva e idade e crescimento deverão ser utilizados no manejo de *P. paru*. Algumas medidas de manejo, relacionadas ao aquarismo, já são aplicadas em *P. paru*. Nos Estados Unidos, o ordenamento da pesca desta espécie para fins ornamentais é realizado por meio da regulamentação de tamanho. No Brasil, o ordenamento é feito com cotas de exportação específicas. Estas regulamentações são fundamentais e ajudam na conservação dos estoques e na prevenção contra a sobrexploração. Entretanto, essas medidas foram tomadas sem nenhum conhecimento dos parâmetros populacionais da espécie, como crescimento, longevidade, idade e tamanho de primeira maturação sexual. Esses parâmetros são requisitos mínimos para um manejo pesqueiro efetivo de uma determinada espécie. Diante do exposto, o manejo de *P. paru* deve incluir medidas baseadas nos parâmetros populacionais aqui estabelecidos, antes que mais restrições sejam impostas. Entretanto, esses parâmetros aqui estabelecidos não se adequam ao manejo das espécies utilizadas no aquarismo, pois a maioria dos indivíduos comercializados é juvenil e inferior a 10 cm de comprimento padrão. Desta forma, sugere-se que as medidas conservacionistas estejam relacionadas a dados quantitativos (captura) por área e criação de áreas de proteção ambiental.

Somada à pressão pesqueira sofrida por esta espécie através do aquarismo, *P. paru* é constantemente capturada como *bycatch* em armadilhas de pesca no nordeste do Brasil. Nesta pescaria, mais de 70% dos indivíduos capturados estavam aptos para a reprodução. É possível que essa espécie sofra pressão pesqueira em todas as faixas de comprimento, uma vez que indivíduos menores que 10 cm são comumente utilizados no aquarismo e aqueles com comprimento entre 15 e 40 cm são frequentemente pescados em armadilhas. A constante captura de indivíduos de maior porte representa um possível risco ao recrutamento para o estoque reprodutivo.

No caso da pesca com armadilhas, não há nenhum tipo de regulamentação para esta atividade. O manejo desta atividade é difícil, por ser considerada multi-específica e com impacto desconhecido. A maioria das artes de pesca não é seletiva e muitos peixes são capturados como *bycatch*, mesmo quando há apenas uma espécie alvo. Esses peixes são altamente afetados, pois enquanto for viável capturar a espécie alvo, o *bycatch* continuará a ser pescado.

A metodologia estabelecida nesta tese é uma ferramenta prática e apropriada para o manejo pesqueiro e avaliação da sustentabilidade da pesca de espécies que são capturados em pescarias multi-específicas e com poucos dados disponíveis sobre o impacto da atividade. Este método também pode ser empregado para determinar quais as espécies estão em um possível risco de extinção devido ao *bycatch*. Além disso, faz-se necessária uma análise de vulnerabilidade sócio-econômica a fim de desenvolver um programa institucional apropriado para os pescadores, no intuito de resolver essa problemática ecológica.

APÊNDICE

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A rapid new method for assessing sustainability of ornamental fish by-catch from coral reefs

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Abstract. The ornamental fish trade has considerable potential for damaging reef environments. Nearly 120 species are currently harvested in the Brazilian trade and little attention has been paid to the effects of the accidental catch of these species. Records of ornamental fish by-catch and the susceptibility and resilience of species from the major Brazilian families in the aquarium trade were examined to test a method and determine the sustainability of these catches. Each species was assessed based on two groups of criteria: (1) relative vulnerability to capture; and (2) population resilience to mortality due to fishing efforts. Each species was also ranked (1–3) for criteria regarding ecology, biology, catchability, numbers of individuals exported by aquarium companies and mortality index. These criteria were then weighted and each species rank in the two groups of criteria was plotted. Nineteen species from 10 families were recorded. The species least likely to have sustainable catch rates included *Holacanthus tricolor*, *H. ciliaris*, *Chaetodon striatus* and *Pomacanthus paru.* This approach is a useful alternative for management efforts and the assessment of catch sustainability in newly developed multi-species fisheries with limited available data, which occur in areas of high species richness such as reefs.

Additional keywords: aquarium, Chaetodontidae, fishery, Pomacanthidae, resilience, susceptibility, trap.

Introduction

Species classified as by-catch (Eayrs 2007) are caught in fisheries that target other groups and are then either used or discarded. It is estimated that 7.3 million tonnes of fish were discarded globally in commercial fisheries between 1995 and 2005 (Kelleher 2005). This is equivalent to 8% of worldwide marine fish production (Eayrs 2007). The global effect of fisheries on by-catch species is poorly known, although some studies have documented ecological changes at the species level (Casey and Myers 1998; Pope *et al*. 2000). Even less attention has been paid to accidental catches affecting reef fish, including ornamental species.

Beauty and perfect patterns on ornamental reef fish are requirements imposed by the international aquarium trade (Araújo and Albuquerque Filho 2005), which is one of the world's largest industries. The import value worldwide of marine fish and invertebrates is estimated to be between US$24 and 40 million annually (Wood 2001*a*). Nearly 120 species are currently harvested in the Brazilian aquarium trade (Monteiro Neto *et al*. 2003). Most belong to the family Pomacanthidae (Araújo and Albuquerque Filho 2005) and species of the Chaetodontidae are among the most exported (Monteiro Neto *et al*. 2003; Nottingham *et al*. 2000). Although other fisheries that use traps have an effect on ornamental marine fish stocks (through by-catch), these stocks have only been managed through a Federal Act (56/04) established in 2004 by the Brazilian environmental protection agency (Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis – IBAMA), which monitors the ornamental fish trade through export quotas. The purpose of this Federal Act is to administer this activity through the establishment of export quotas.

Traps are the predominant gear employed to capture reef fish in the Caribbean (Swingle *et al*. 1970; Garrison *et al*. 1998) as well as other places in the world (e.g. Arabian Gulf, Seychelles, Papua New Guinea) (Dalzell *et al*. 1996) and are responsible for the decline of many reef species which are fishery targets (Wolff *et al*. 1999). Among the organisms caught by these devices, the most frequently caught ornamental fish belong to the following genera: *Acanthurus*, *Chaetodon*, *Bodianus*, *Holacanthus* and *Pomacanthus* (Garrison *et al*. 1998; Wolff *et al*. 1999). In Brazil, trap fisheries targeting reef fish have been on the rise in the last 10 years (Ribeiro 2004). The target species of these fisheries belong to the genera *Acanthurus*, *Cephalopholis*, *Lutjanus*, *Pseudupeneus* and *Sparisoma* (Ribeiro 2004). Because by-catch species, including ornamental species, are returned to the sea and not recorded, there are no data or regulations available about the actual catch of ornamental species.

Owing to the increasing pressure to manage the impact of fisheries on non-target species and the uncertainty of how this is to be done, Stobutzki *et al*. (2001) developed a broad method using qualitative measures of the biological and ecological characteristics of species to rank vulnerability to a known threat. Stobutzki *et al*. (2001) examined the impact of trawling on the catch sustainability of teleost by-catch species, identifying the species with the least likelihood of catch sustainability in by-catch. They argued that such species should then be the focus

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of research and management. Stobutzki *et al.* (2001) recorded at least 411 species from 99 families as by-catch, identifying 15 species with the least likelihood of catch sustainability as well as the 13 with the most likelihood of catch sustainability. This approach was designed to overcome problems such as the high diversity of by-catch in tropical fisheries and the lack of information on individual species. The method is versatile and can also be applied to other fisheries, although the criteria employed may vary.

The present study describes a rapid, innovative method, based on the method developed by Stobutzki *et al.* (2001), for evaluating the impact of trap fisheries on ornamental species using biological data and the portion of the population caught. The intention is to answer the following two questions. (1) Is the method appropriate for ornamental reef fish? (2) Is the capture of ornamental reef fish as by-catch sustainable? The present study records the occurrence of ornamental fish caught as by-catch and assesses the susceptibility and resilience of fish from the Pomacanthidae and Chaetodontidae families.

Materials and methods

Sampling

The fish were acquired from the fishing colony of Itamaracá island located on Pilar Beach (7°45′17.80″S, 34°49′26.46″W) (Itamaracá, Pernambuco, Brazil – Fig. 1) and were caught as by-catch while trap fishing for the spotted goatfish, *Pseudupeneus maculatus* and parrotfish, *Sparisoma* spp. This kind of fishing occurs throughout the year and is carried out on alternating days. The boats leave Pilar Beach at midnight and arrive at Tambaba Beach, 46 km away (7°22′2.93″S, 34°47′48.51″W) (Paraíba, Brazil) in the early morning. Four boats are employed in this fishery. Each has a fishing effort of 15 to 20 traps and trap mesh varying between 1 and 7 cm. The traps are deployed unbaited next to reefs and hauled 6 h later. Occasionally, depending on factors such as wind and currents, some traps are left for longer periods and only hauled after 48 h. The fish are stored for ~7 h in 100-L containers. For the present study, weekly samplings were performed from April to June 2006. The fishermen were asked to bring back all specimens with ornamental characteristics, which were then bought. In the case of *Holacanthus ciliaris* and *Pomacanthus paru*, this procedure was carried out over a longer period (March 2006 to February 2007), as these two species were the focus of a concurrent study.

The sampled fish were placed in polystyrene chests with ice and taken to the Nekton and Aquaculture Laboratory of the Oceanography Department of the Universidade Federal de Pernambuco, where they were identified, measured and photographed.

Analysis of catch sustainability of ornamental reef fish by-catch

Only the most abundant species were analysed, i.e. species from the Pomacanthidae and Chaetodontidae. Rare species for Brazil, such as *Chaetodon ocellatus* and *Pomacanthus arcuatus* (Thresher 1980; Carvalho Filho 1999) were not surveyed owing to the small sample size. Information on the biological characteristics of the selected species was taken from published literature including Robins and Ray (1986), Thresher (1980, 1984), Allen (1998), Hourigan *et al.* (1989) and Froese and Pauly (2007).

The method proposed to assess the sustainability of selected by-catch species was adapted from Stobutzki *et al.* (2001) and applied based on two scored sets of criteria: (1) relative vulnerability to capture by traps and mortality due to trap fishery; and (2) the recovery capacity of a species after the population is depleted. The criteria were originally developed during a study on the sustainability of all North Australian Prawn Fishery (NPF) prawn-trawl vertebrate by-catch (Stobutzki *et al.* 2000). These criteria were modified and assigned relative importance weights by the NPF Assessment Group (Milton 2001). Values ranging from 1 to 3 were attributed to each sustainability indicator, for which 1 denotes the highest value for the susceptibility criteria and the lowest for resilience. Depending on the criterion, these values were based on either categorical or continuous data. When no information was available to assign the divisions between values, the range of the data was divided into thirds to create categories (Table 1). The rank matrix constructed from these values for each set of criteria are plotted as axes (resilience versus susceptibility) on a graph indicating the species with the greatest catch sustainability. This graph is recommended only if the different species have different values on each axis. According to Stobutzki *et al.* (2001), when species-specific information is unavailable, a species is given the same value as other species within the same family. With criteria for which family members are not similar or for which no family information is available, the rank of 1 is given as a precautionary measure.

The criteria adopted to analyse species susceptibility (axis 1) to capture and possible mortality due to fishery efforts were adapted from Stobutzki *et al.* (2001) and are summarised as follows. (1.1) Water column position – as traps are deployed on the substrate (Mahon and Hunte 2001; Garrison *et al.* 2004), benthic and demersal species are more susceptible to capture than pelagic species. (1.2) Preferred habitat – species are ranked according to their preference for rocky habitats. This criterion addresses the likelihood that a species habitat overlaps the area in which a trap fishery occurs. Coral reef habitats have been historically targeted by trap fisheries (Wolff *et al.* 1999). Thus, species that live near shore and depend on a specific habitat, such as coral reefs, are highly vulnerable to a possible risk of extinction (Dye *et al.* 1994; Roberts and Hawkins 1999). (1.3) Day/night catchability – because trap fishery is a daytime operation, species with diurnal habits are more susceptible to capture by traps. (1.4) Diet – whether eating habits attract species to trap fishery areas. Thus, species that generally feed on benthic or demersal organisms found on reefs are more vulnerable to capture. Invertebrate feeders (mobile and sessile) are the dominant trophic guild in traps deployed in coral reef habitats (Garrison *et al.* 2004). (1.5) Depth range – the trap fishery that we studied occurs in depths between 30 and 40 m. According to Wolff *et al.* (1999), trap fisheries in adjacent coral reef and gorgonian habitats range from 3 to 38 m. Overlap between the depth range of the trap fishery area and that of the preferred habitat will affect the vulnerability of a species to capture more than that of others that live in either deeper or shallower areas. Fish that have a narrow vertical depth range are highly vulnerable to extinction (Dye *et al.* 1994; Roberts and Hawkins 1999) (Table 1).

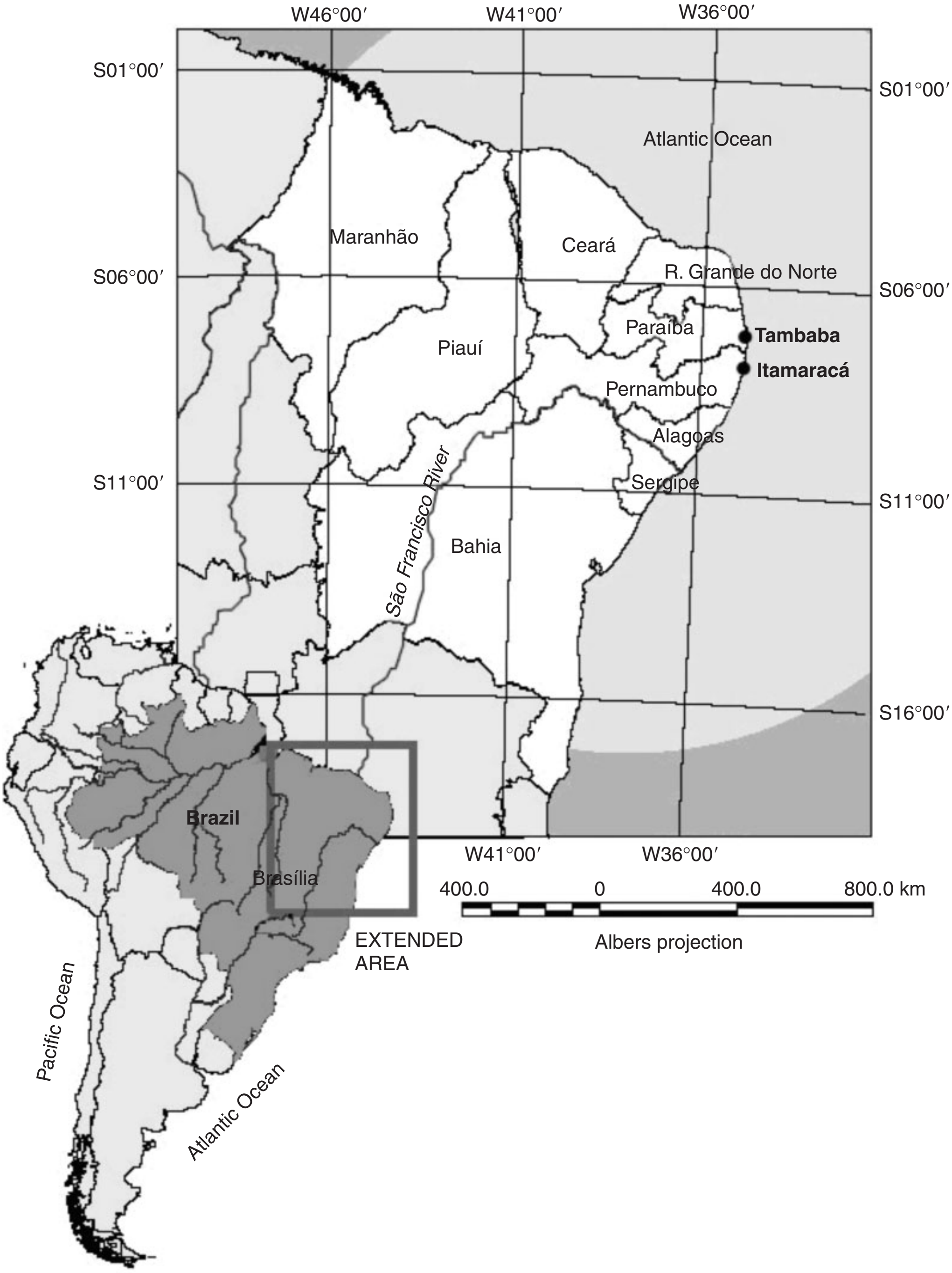

Fig. 1. Location of sampled area.

Six criteria adapted from Stobutzki *et al*. (2001) were employed to analyse species resilience. (2.1) Maturity – if most individuals caught are larger than their size at first maturity, the species has a greater probability of recovering from a declining population. (2.2) Maximum size – species that attain large sizes tend to live longer and therefore the resilience of the population is lower than species with smaller body sizes. A slow life history (e.g. late maturity, long reproductive span and large body size) is related to low maximum population growth rates and, consequently, such species are more vulnerable and have less capacity to recover from exploitation (Hutchings 2001; Reynolds *et al.* 2001, 2002). (2.3) Removal rate – species-based quotas established by IBAMA to ensure that harvests are maintained at a sustainable level. The ornamental species fishery is highly selective and certain species, such as chaetodontids and pomacanthids, are preferred targets. Among those, the species that are rare may be vulnerable to over-exploitation (Wood 2001*b*). (2.4) Reproductive strategy – species capable

Table 1. The criteria used to assess the susceptibility of by-catch species to capture and mortality due to traps and their recovery
For each criterion the weighting score and definition of the three ranks is given

Criteria	Weighting score	1	2	3
Susceptibility				
Water column position	3	Demersal or benthic	Benthopelagic	Pelagic
Preferred habitat	3	Rocky habitats with gravel and sand	Habitats with soft or muddy bottoms	No fishing habitats
Day/night catchability	2	Higher catch rate at day	No difference between day and night	Higher catch rate at night
Diet	3	Feed on demersal or bent. organisms	Feed on benthopelagic organisms	Feed on pelagic organisms
Depth range	1	Less than 40 m	–	Deeper than 40 m
Resilience				
Maturity	3	Mature individuals $<50\%$		Mature individuals $>50\%$
Maximum size	3	Max. size ≥ 50 cm	$16\text{ cm} <$ max. size < 50 cm	Max. size ≤ 16 cm
Removal rate	3	Catch ≥ 3500 inds/comp/yr	$1000 <$ catch < 3500 inds/comp/yr	Catch ≤ 1000 inds/comp/yr
Reproductive strategy	2	Bear live young or brood young	Guard eggs and/or young	Broadcast spawners
Hermaphroditism	1	Protandric hermaphrodites	Protogynous hermaphrodites	Dioecious
Mortality index	1	Mortality index ≥ 3.19	$0.96 <$ mortality index < 3.19	Mortality index ≤ 0.96

of dispersal over long distances are less vulnerable to extinction (Dye *et al.* 1994; Roberts and Hawkins 1999). Thus, the resilience of R-strategist species is generally higher than that of K-strategist species. (2.5) Hermaphroditism – hermaphroditic species, especially protandric species, may be at great risk of extinction because fisheries select individuals of certain sizes and hermaphroditic fish generally only change sex when they reach a certain size, so the fishing effort is, in effect, directed at only one sex – which can have a negative effect on reproduction success (Dye *et al.* 1994; Roberts and Hawkins 1999; Hawkins and Roberts 2004). (2.6) Mortality index – this index was proposed by Sparre and Venema (1992) and Stobutzki *et al.* (2001). This index can be derived from the length–frequency of a species and von Bertalanffy growth parameters. Such parameters are not available for most by-catch species, but the index can be calculated as follows:

$$\text{Mortality index } (Z)\text{: } (L_{\max} - L_{\text{mean}})/(L_{\text{mean}} - L_{\min})$$

in which $L_{\max}$ is the maximum length recorded in the literature, L_{mean} is the mean length of total number of individuals caught and $L_{\min}$ is the minimum length of the individuals in the sample. In the present study, the mortality index range was calculated (0.13–0.74) and divided into thirds for the division between ranks (Table 1).

Analysis of criteria

The overall ranks of susceptibility or resilience were calculated according to the equation below:

$$S_i = \sum_{j=1}^{n} R_i \bigg/ \sum_{j=1}^{n} w_j$$

in which S_i is the total susceptibility or resilience rank for species i, w_j is the weight for criterion j; R_i is the rank of species i for criterion j and n is the number of criteria for each axis.

Statistical analysis

Data normality was evaluated using the Kolmogorov–Smirnov test (BioEstat 5.0 software – Instituto de Desenvolvimento Sustentável Mamirauá – Amazonas). The parametric Z-test (BioEstat 5.0 software) was used to determine the probability of a captured individual being smaller than size at first maturity and the probability of a captured individual having bred at least once. Correspondence analysis (Multivariate Statistical Package – MVSP 3.1 software – Kovach Computing Services – Anglesey, wates) was used to determine and describe the correspondence between species and criteria on the same graph. This multivariate analysis was used only for criteria related to resilience because values regarding susceptibility did not vary among species. Since the variance of the reproductive strategy criterion was equal to zero, this criterion was not applied in the present analysis.

Results

Among the fish caught, 19 were recorded as by-catch species and belonged to 14 genera and 10 families (Table 2). Of these, 100% were from reef environments and are used in the aquarium trade and only one species (*Equetus lanceolatus*) was not authorised for export. In total, 430 individuals were sampled: 147 *Holacanthus ciliaris*, 46 *Holacanthus tricolor*, 200 *Pomacanthus paru* and 37 *Chaetodon striatus*. Length–frequency distribution by species (Fig. 2) revealed a broad length range among the individuals sampled.

All susceptibility criteria are species-specific and the four species evaluated in the sustainability analysis obtained the same value (1) for this criterion. Rank resilience ranged from 1.92 to 2.54; *H. tricolor* and *P. paru* obtained the smallest and the largest values respectively (Table 3). Based on these results, it can be affirmed that *H. tricolor* has the least catch sustainability, followed by *H. ciliaris* and *C. striatus*. *P. paru* is the most capable of recovering from a population decline.

Mean and smallest lengths for *H. tricolor* and *C. striatus* individuals were under size-at-first-maturity. There was a 71% probability of *H. tricolor* individuals and a 92% probability of *C. striatus* being caught at sizes smaller than first maturity. Contrarily, the probability of captured individuals being larger than maturity size was 76% for *H. ciliaris* and 70% for *P. paru*. *H. ciliaris* had the highest mortality index ($Z = 3.19$) and *P. paru* had the lowest ($Z = 0.96$) (Table 4).

Table 2. Species registered as by-catch, with the respective common names

Family	Species	Common name
Sygnathidae	*Hippocampus reidi* Ginsburg, 1933	Longsnout seahorse
Synodontidae	*Synodus foetens* (Linnaeus, 1766)	Inshore lizardfish
Aulostomidae	*Aulostomus maculatus* Valenciennes, 1841	Trumpetfish
Sciaenidae	*Equetus lanceolatus* (Linnaeus, 1758)	Jack-knife fish
Sciaenidae	*Pareques acuminatus* (Bloch & Schneider, 1801)	Highhat
Chaetodontidae	*Chaetodon ocellatus* Bloch, 1787	Spotfin butterflyfish
Chaetodontidae	*Chaetodon striatus* Linnaeus, 1758	Banded butterflyfish
Pomacanthidae	*Holacanthus ciliaris* (Linnaeus, 1758)	Queen angelfish
Pomacanthidae	*Holacanthus tricolor* (Bloch, 1795)	Rock beauty
Pomacanthidae	*Pomacanthus arcuatus* (Linnaeus, 1758)	Gray angelfish
Pomacanthidae	*Pomacanthus paru* (Bloch, 1787)	French angelfish
Labridae	*Bodianus rufus* (Linnaeus, 1758)	Spanish hogfish
Labridae	*Bodianus pulchellus* (Poey, 1860)	Spotfin hogfish
Labridae	*Halichoeres dimidiatus* (Agassiz, 1831)	Brazilian yellowcheek wrasse
Labridae	*Halichoeres brasiliensis* (Bloch, 1791)	Brazilian wrasse
Monacanthidae	*Cantherhines macrocerus* (Hollard, 1855)	Whitespotted filefish
Monacanthidae	*Stephanolepis hispidus* (Linnaeus, 1766)	Planehead filefish
Ostracidae	*Lactophrys trigonus* (Linnaeus, 1758)	Buffalo trunkfish
Tetraodontidae	*Canthigaster figueiredoi* Moura & Castro, 2002	Southern Atlantic sharpnose-puffer

The correspondence analysis for resilience revealed that *H. tricolor* and *C. striatus* are linked to the removal index. *H. ciliaris* and *P. paru* are associated through maturity criteria. *C. striatus* and *P. paru* are grouped with maximum size and mortality index criteria respectively. Negative relationships were determined for: *H. ciliaris* and the removal rate and mortality index; *H. tricolor* and *C. striatus* with maturity criteria; and *P. paru* with maximum size. The percentage of the cumulative variation for the two axes was 99.53% (Fig. 3).

Discussion

The present study demonstrates that the trap fishery is not selective. The catching of ornamental fish occurs frequently and likely causes considerable impact. Ornamental species are already considered seriously endangered owing to fishery efforts (Cheung *et al.* 2007). Individuals caught are not landed and are usually returned to the sea (many still alive, according to the fishermen). Despite this, the survival of these individuals is doubtful, as the traps are hauled quickly to surface and problems related to decompression have often been reported (Burns and Restrepo 2002).

The method applied in the present study was developed with the purpose of classifying ornamental fish species according to possible risk of extinction owing to being captured as by-catch. This analysis is influenced by the length range and proportion of young and immature individuals sampled because criteria that are directly related to specimen size are employed, such as length at first maturity and mortality index. Different mesh sizes are used in this fishery, which may have contributed to the broad length range among the individuals sampled. Owing to the small sample size for *H. tricolor* and *C. striatus*, the length range of the specimens caught may not be representative. Thus, for these species, the analysis must be considered only for demonstration (exploratory) purposes, even though there was a trend towards normality in the length distribution data. The present study is the first to test a method with such goals directed at trap fisheries and using criteria based on life history.

Specific biological information is optimised when well defined criteria are applied, including characteristics that affect species sensitivity or risk of extinction (Dye *et al.* 1994; Roberts and Hawkins 1999; Stobutzki *et al.* 2001). Stobutzki *et al.* (2001) applied the value 1 in the matrix when there was no specific information for certain criteria. In the present study, all biological information was species-specific. Although the analysis presented is subjective, this may be the only one available to evaluate and monitor multi-specific fisheries (Dulvy *et al.* 2003), especially newly developed fisheries.

Susceptibility to capture by traps

Species caught by a given equipment share similar characteristics, such as habitat, diet and period of activity. Thus, reef fish are considered highly susceptible to trap fisheries. Species of Pomacanthidae are widely distributed in depths ranging from 1 to 100 m (Allen 1998; Michael 2004; Froese and Pauly 2007), whereas species of Chaetodontidae occur between 3 and 55 m (Robins and Ray 1986; Allen 1998; Froese and Pauly 2007). The former feed on sponges, zoanthids, tunicates, hydroids, bryozoans and algae (Randall 1967; Allen 1998; Michael 2004). Chaetodontids eat worms, coral polyps and mollusc eggs (Allen 1998). The diet and period of activity of these fish favour their capture because they only feed once during the day (Starck and Davis 1966) and the organisms they eat are sessile, which are generally encrusted on reefs. Dependence on a particularly susceptible habitat (e.g. coral reefs), either alone or combined with overfishing, leads to considerable captures of by-catch species and makes these species all the more vulnerable (Reynolds and Jennings 2000).

Resilience

Species with late sexual maturity are highly vulnerable to the risk of extinction and consequently have a poor recovery capability

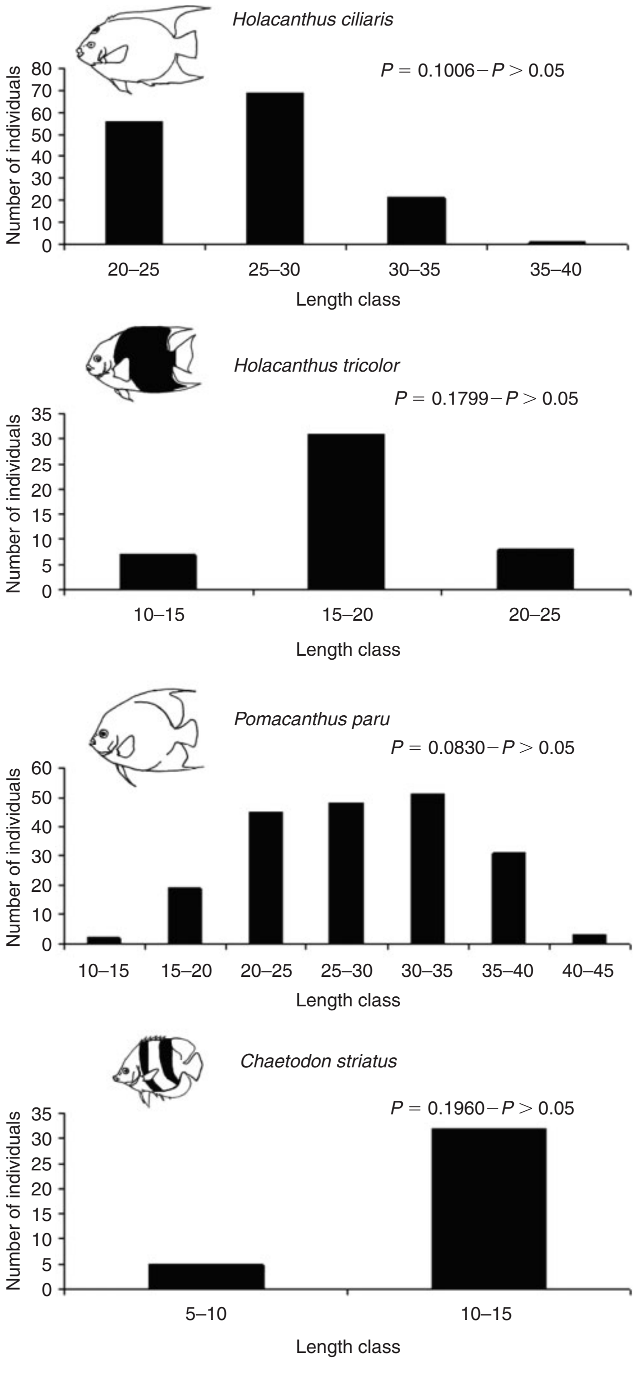

Fig. 2. Length distributions for the four analysed species.

(Dye *et al.* 1994; Roberts and Hawkins 1999; Sadovy 2005). The correspondence analysis reveals the clustering of *H. ciliaris* and *P. paru* with the maturity criterion owing to the fact that more than a half of the sampled individuals had bred at least once. The opposite was found for *H. tricolor* and *C. striatus*, for which, respectively, 70% and 92% of individuals sampled were immature. Myers and Worm (2005) stated that the risk of extinction is very high if recruits are being captured and low when fish are caught after first sexual maturity. Thus, the maturity criterion had a positive influence on the classification of *H. ciliaris* and *P. paru* in relation to resilience.

Large body size is often correlated with other vulnerability factors, such as late maturity, and substantially increases the possibility of being caught in traps (Reynolds and Jennings 2000; Reynolds *et al.* 2002; Cheung *et al.* 2007). Body size is a useful measure in evaluating species resilience owing to its relationship with the intrinsic rate of increase of a population. Thus, it can be applied to populations and species for which minimal biological data are available (Denney *et al.* 2002). In the Pomacanthidae, the genera *Holacanthus* and *Pomacanthus* are the longest (Bellwood *et al.* 2004). Among the analysed fish, *P. paru* was the second largest, but exhibited the greatest resilience. This can be explained by the fact that most individuals caught were fit for reproduction and the species had the lowest mortality rate.

The demand for marine ornamental fish increased in the 1980s and 1990s (Chan and Sadovy 1998). Concerns have been raised regarding its negative effect on reef fish and coral habitats because nearly all ornamental marine fish are taken from the wild (Andrews 1990; Wood 2001*a*). The potential for overexploitation from the aquarium trade is high owing to the high selectivity of this activity and the large number of fish collected (Wood 2001*a*). It is estimated that most commercialised ornamental fish are juveniles and less than 10 cm in standard length (Chan and Sadovy 1998). Species of Pomacanthidae and Chaetodontidae are often the most important families for export in terms of number and value (Wood 2001*a*). In the Brazilian aquarium trade, these two families are among the most representative in species richness (Gasparini *et al.* 2005). In the state of Ceará (one of the main centres of the Brazilian ornamental fish trade), *H. ciliaris*, *P. paru*, *Hippocampus erectus*, *Pomacanthus arcuatus* and *Holacanthus tricolor* made up 50% of the total trade between 1995 and 2000 (Monteiro Neto *et al.* 2003). Among the species evaluated, *H. ciliaris* is the most harvested by the Brazilian aquarium trade (IBAMA – Federal Act 56/04). The correspondence analysis corroborates this statement, revealing a negative relationship between *H. ciliaris* and removal rate.

Regarding reproductive strategy, all species evaluated are broadcast spawners (Thresher 1980, 1984). Therefore, they are less vulnerable to extinction owing to their widespread distribution and the high recolonisation potential of depleted populations (Malakoff 1997; McKinney 1997). However, larval behaviour, spawning events and local oceanography processes can influence larval dispersal by increasing local retention (Cowen *et al.* 2006; Rocha *et al.* 2007). Hawkins *et al.* (2000) stated that, despite reef fish being broadcast spawners, their geographic distribution is small: 24% are restricted to less than 800 000 km^2 and 9% are restricted to less than 50 000 km^2. Based on these statements, it is possible to infer that being a broadcast spawner does not ensure low vulnerability to extinction.

Hermaphroditic species are likely to be very sensitive to overfishing because individuals need to attain a certain length for the sex change to occur (Francis 1992). If fishing affects the growth of the fish such that sexual inversion does not occur, this could result in a non-reproductive population and even cause the species to disappear in intensely exploited areas (Hawkins and Roberts 2004). Among the species analysed in the present study, those from the *Holacanthus* genus are protogynous hermaphrodites (Thresher 1980, 1984; Moyer *et al.* 1983;

Table 3. The ranking of the species on the criteria on the resilience axis

Species/weight	Resilience						
	3	3	3	2	1	1	Rank
	Maturity	Maximum size	Removal rate	Reproductive strategy	Hermaphroditism	Mortality index	
Holacanthus ciliaris	3	2	1	3	2	1	2.08
Holacanthus tricolor	1	2	2	3	2	2	1.92
Pomacanthus paru	3	2	2	3	3	3	2.54
Chaetodon striatus	1	3	3	3	3	2	2.46

Table 4. The length at first capture, length at maturity, probability that individuals caught are below the length at maturity, probability of breeding, mean and maximum length (max. length) of the species and the mortality index (*Z*) of four species of fish

Species	*n*	L′ (cm)	Lm (cm)	Prob. L′ < Lm (cm)	Prob. of breeding	L_{mean} ± s.e. (cm)	Max. length (cm)	*Z*
Holacanthus ciliaris	147	20.88	24.3	$P = 0.24$	$P = 0.76$	26.58 ± 0.2672	45 (Allen 1998)	3.19
Holacanthus tricolor	46	11.32	19	$P = 0.71$	$P = 0.29$	17.61 ± 0.3646	35 (Allen 1998)	2.68
Pomacanthus paru	200	13.44	25	$P = 0.30$	$P = 0.70$	28.30 ± 0.4510	41 (Cervigón 1993)	0.96
Chaetodon striatus	37	9.09	13.2	$P = 0.92$	$P = 0.07$	11.26 ± 0.2257	16 (Lieske and Myers 1994)	2.17

n = number of individuals, L′ = length at first capture, Lm = length at maturity, Prob. = probability.

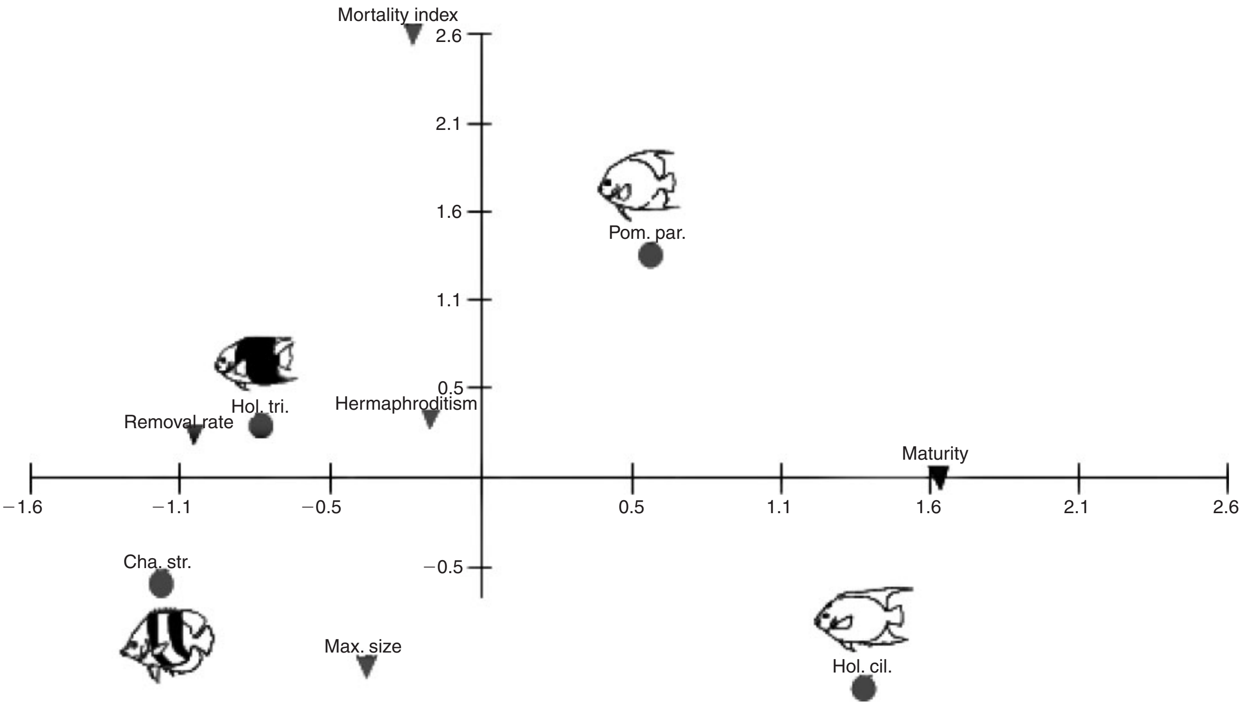

Fig. 3. Correspondence analysis showing the relationship between species and criteria used to evaluate the recovery capacity. Hol. cil. = *Holacanthus ciliaris*, Hol. tri. = *Holacanthus tricolor*, Cha. str. = *Chaetodon striatus*, Pom. par. = *Pomacanthus paru*, Max. size = maximum size.

Allen 1998). These species are heavily exploited by the aquarium trade, in which most individuals collected are small in size (Chan and Sadovy 1998) and probably belong to the same sex (i.e. female). *H. tricolor* and *H. ciliaris* revealed the lowest recovery capacity. However, according to the correspondence analysis, the hermaphroditism criterion had no influence over their classification regarding resilience. This may be explained by the absence of protandric species in the analysis because such species are more sensitive than protogynous species (Sadovy and Shapiro1987; Hawkins and Roberts 2004).

Throughout the life cycle, individuals are exposed to different types of environmental pressure. The natural mortality rate decreases as the fish grows and becomes less vulnerable to predators. The mortality coefficient used in the present study considered data on maximum and mean catch length (Beverton and Holt 1956). *H. ciliaris* had the highest mortality index among the species analysed owing to the mean catch size, which was below that found in the literature (Allen 1998). This coefficient – along with the criterion related to the removal rate – had a negative influence on the resilience of this species.

Resilience ranks were different for the four species analysed, even for those belonging to the same family. Stobutzki *et al.* (2001) stated that the catch of a species is not sustainable if it has both high resilience and susceptibility. The results obtained for *C. striatus* and *P. paru* are in agreement with this statement because these species revealed considerable resilience, but were highly susceptible according to the ecological information (such as diet, preferred habitat and depth range).

The method employed in the present study (susceptibility and resilience criteria) is efficient for evaluating the impact of newly formed fisheries with few available data that occur in areas with high species richness (Dulvy *et al.* 2003, 2004), such as reef environments. However, in order to incorporate the results of this study in fishery management measures, vulnerability analysis (VA) can be utilised as a component of a risk analysis when only qualitative data is available and researchers lack an understanding of cause/effect relationships (Walker *et al.* 2002; McFadden 2007).

Most fishing equipment is not selective and many fish are caught as by-catch, even when there is only one target species. These species are highly affected by fishery efforts because by-catch will continue to be fished as long as it remains viable to catch the target species. Trap fisheries targeting reef fish have recently developed in Brazil and are on the rise in the north-eastern region. Regulatory agencies often take a long time to react and severe declines in non-target species go unperceived. Thus, little is known regarding the impact of this activity and such lack of information hinders regulatory efforts. The method presented here is an appropriate, practical tool for fishery management and the assessment of catch sustainability in multi-species fisheries on which there is limited available data. It can also be employed to determine which species are at a possible risk of extinction owing to being by-catch. Furthermore, it is important to consider that to reduce the extinction threat posed on those species, a socioeconomic VA is required in order to develop the appropriate institutional framework/outreach program to the fishers necessary to address this ecological challenge.

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