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Ana Santos Ferreira

Dissertação

Mestrado em Biologia da Conservação

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Orientadores:

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Núria Baylina

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Summary

Elasmobranchs are among the most threatened marine species at a global scale, mostly affected by over-fishing and habitat loss. It is now recognized the urgent need to adopt effective measures for *in situ* and *ex situ* conservation of these animals. The ribbontail stingray, *Taeniura lymma* (Forsskål, 1775) is a very attractive species for public aquariums. It is classified as "near threatened" by the IUCN and considered a strong candidate for a captive breeding program. In 2007, the European Studbook for *T. lymma* was implemented, to monitor the status and trend of the captive population, being coordinated by Oceanário de Lisboa. This study describes the establishment of a breeding group through monitoring, target feeding and animal training, for desensitization to clinical exams and biometric data collection. Environmental and water quality parameters were defined and controlled, to promote the welfare of breeding adults and juveniles. Successful breeding was achieved and pregnant females were monitored until the end of gestation. Fifteen rays were born with litters ranging from one to two pups. Gestation period ranged from 128 to 203 days. The neonates were subjected to a feeding and data collection protocol to assess their growth. No significant differences were found in the birth size and weight, between sexes or litter sizes. Growth in weight fitted a linear regression model and a growth of $3.25 g.day^{-1}$ rate was estimated. The individuals weight increase after thirty, sixty and ninety days was also significantly different however, it could not be associated with the juveniles gender or litter size. This study intends to be a tool for replication of such programs in other institutions that keep this species. It will also increase the knowledge of this species biology and might contribute to the future establishment of effective conservation actions.

Key words: *Taeniura lymma*, captive breeding, monitoring and training, growth assessment; elasmobranchs conservation

Resumo

Os oceanos estão em risco de entrar numa fase de extinções de espécies, sem precedentes na história humana. A sobre exploração e a degradação de habitats são as principais causas de declínio das populações. Para fazer face a esta problemática global, diversos países têm trabalhado em conjunto, no sentido de definir e aplicar medidas eficazes de conservação das espécies e dos habitats nos ecossistemas do nosso planeta. É atualmente reconhecida a importância de medidas de conservação *in situ* e *ex situ* para estes animais. Os Elasmobrânquios são um grupo particularmente vulnerável à sobre exploração, devido às suas características de vida nomeadamente, o crescimento lento, a maturação sexual tardia e a baixa fecundidade. Os *Chondrichthyans*, estão entre as espécies marinhas mais ameaças, sendo alvo de diversos tipos de pesca, comercial, artesanal e acessória, sendo os *Carcharhinids*, *Shpyrnids* e *Batoids*, os grupos mais afectados. A espécie *Taeniura lymma* (Forsskal, 1775) ou uge-de-pintas-azuis, é uma espécie muito procurada para exibição em Aquários Públicos. A sua distribuição geográfica ocorre nos oceanos Índico e Pacífico e o seu habitat característico são os recifes de coral. Caracteriza-se por um padrão único para cada indivíduo, de pintas azuis distribuídas por toda a parte dorsal, sendo a parte ventral de coloração esbranquiçada. É uma espécie frequentemente capturada como espécie acessória, por artes de pesca comerciais e artesanais, uma vez que é pouco procurada para consumo humano e o seu valor comercial não é elevado. Esta espécie é considerada como "quase ameaçada" pela União Internacional para a Conservação da Natureza e dos Recursos Naturais. As principais ameaças que enfrenta são a degradação do seu habitat, a pressão exercida pela pesca artesanal, pesca acessória e comercial e a elevada procura pelo mercado de aquariofilia. Trata-se de uma espécie de difícil manutenção em cativeiro, uma vez que é muito vulnerável ao stress inerente ao seu maneio. São animais bastante seletivos no que diz respeito à alimentação sobretudo, durante o período de transição entre o ambiente natural e o de cativeiro. Até ao presente, poucas instituições europeias conseguiram com êxito a reprodução desta espécie em ambiente controlado. Em termos de reprodução, são animais vivíparos aplacentários, com baixa taxa de reprodução, longo período de gestação (de 4 a 12 meses) e longevidade desconhecida. Devido a estas características, esta espécie é uma forte candidata para o desenvolvimento de programas de reprodução em cativeiro. Em 2007 foi implementado o *European Studbook*, desenvolvido pelos membros da *European Association for Zoos and*

Aquariums e coordenado pelo Oceanário de Lisboa. Esse programa pretende desenvolver a reprodução destes animais em cativeiro, aumentando o número de instituições europeias que possam manter grupos reprodutores e a avaliação do estado da população mantida em cativeiro, com o objetivo de diminuir o número de espécimes capturados em meio natural para exibição em Aquários Públicos. Este estudo descreve a monitorização de *T. lymma,* com o objetivo de obter um grupo reprodutor e de estudar pela primeira vez, o crescimento destes animais nascidos em cativeiro. O trabalho foi desenvolvido com um grupo de cinco indivíduos, quatro fêmeas e um macho, provenientes do meio natural e mantidos no aquário do Habitat Tropical, no Oceanário de Lisboa, com 243 mil litros de água, onde coabitaram com diversas espécies de peixes do Indo-Pacífico. Não existiam potenciais predadores para as *T. lymma* no aquário e a sua decoração, recreava um ecossistema de recife de coral, com diversos locais de refúgio para os animais. As raias foram continuamente monitorizadas e alvo de um plano de treino, para permitir a realização de sessões de alimentação individual e dessensibilização a métodos de recolha de dados biométricos e exames clínicos. O treino foi realizado com os cinco indivíduos que constituíam o grupo reprodutor, consistindo em sessões de alimentação, nas quais os animais foram dessensibilizados à presença de um mergulhador e à utilização de um objecto-alvo. Os animais foram recompensados com alimento segundo as suas progressivas aproximações ao objecto-alvo numa primeira fase, até atingirem o objectivo final, de seguirem esse mesmo objecto até ao local escolhido como zona de alimentação. Nessa fase final do treino, as sessões de alimentação foram realizadas a partir da superfície, não sendo necessária a presença de um mergulhador. Esse local, estando numa zona superficial do aquário, possibilitou que os visitantes do aquário presenciassem as sessões de alimentação, permitindo a sua sensibilização para a conservação da espécie e a comunicação do trabalho desenvolvido para esse propósito. A zona escolhida como local de alimentação facilitou o acesso aos animais, permitindo a sua captura regular, para sessões de pesagem e medições, com níveis mínimos de stress para os indivíduos. Os tempos de resposta às várias etapas do treino foram diferentes para as cinco raias mas todas atingiram o comportamento esperado. Numa fase posterior do estudo, o treino com um objecto-alvo foi também executado com as raias juvenis, nascidas em cativeiro, para melhor monitorizar o seu comportamento e alimentação. Os parâmetros ambientais a que os indivíduos estiveram sujeitos, no aquário de exposição e nos aquários de quarentena, foram definidos e controlados, para permitir as melhores condições de

saúde para as raias adultas e juvenis. Foi potenciado o desenvolvimento de um grupo de indivíduos até à maturação sexual e a ocorrência de reprodução. As fêmeas grávidas foram acompanhadas durante a gestação, através de ecografias, para avaliar o seu estado geral, monitorizar os sinais vitais dos embriões em desenvolvimento e identificar o número dos mesmos, em cada gestação. De 2011 a 2013 nasceram quinze crias nas instalações do Oceanário de Lisboa. O período de gestação foi de 128 a 203 dias. Os recém-nascidos foram submetidos a um protocolo específico de nutrição e de recolha de dados biométricos, para o estudo do seu crescimento. Após o nascimento e até aos três meses de idade, as raias foram alimentadas *ad libitum* em sete refeições diárias. Durante este período, novos itens foram adicionados à sua alimentação, para estimular a aceitação de novos alimentos, fornecendo uma alimentação variada e nutricionalmente equilibrada. A frequência de pesagem das raias foi definida e aplicada de acordo com o seu desenvolvimento. Não foram encontradas diferenças significativas de tamanho e peso à nascença, entre machos e fêmeas nem entre crias únicas e gémeas. O crescimento em peso dos indivíduos ajustou-se a um modelo de regressão linear, sendo possível estimar uma taxa de crescimento em peso de 3.25g por dia. O incremento de peso das raias aos 30, 60 e 90 dias de vida revelou diferenças significativas no entanto, estas não puderam ser explicadas pelos diferentes sexos ou número de crias de uma gestação. Este estudo pretende ser uma ferramenta, possível de ser utilizada para replicação de programas semelhantes, noutras instituições, que mantenham esta espécie em cativeiro. A informação recolhida é um contributo para o aumento do conhecimento da biologia das *T. lymma*, podendo ser uma mais valia para a implementação futura de medidas para a sua conservação.

Palavras- chave: *Taeniura lymma*, reprodução em cativeiro, monitorização, treino, estudo de crescimento, conservação.

1. General Introduction

Serious concerns have been raised worldwide about the increasing depletion rate marine fish populations are facing. Chondrichthyans are among the most affected groups, due mostly to overfishing and habitat loss (Stevens et al., 2000; Baum, 2003; White and Dharmadi, 2007; Camhi et al., 2008; Dulvy et al., 2008).

Sharks and rays appear to be particularly vulnerable to over-exploitation due to their life-history strategy, characterized by slow growth, late attainment of sexual maturity, long life spans, low fecundity and natural mortality (Stevens et al., 2000; Camhi et al., 2008; Izzo and Rodda, 2012). Once overexploited a population would take several years to recover (Myers and Worm, 2003; Dulvy et al., 2008). Chondrichthyans are a common but unspecified by-catch in several fisheries (Camhi et al., 2008) and the main groups taken are *Carcharhinids*, *Shpyrnids* and *Batoids* (Stevens et al., 2000). Indonesia has the largest chondrichthyan fishery in the world with estimated annual landings exceeding 100 000t and the proportion of *Batoids* landed has increased almost 20% between 1981 and 2003 (Stevens et al., 2000; White and Dharmadi, 2007). This region also has the highest biodiversity and endemism rates and minimal controls of fishing, suggesting a higher than average extinction risk (Stevens et al., 2000). Of 133 marine vertebrate, invertebrate, and algae populations that have gone locally, regionally, or globally extinct within the last 300 years (Dulvy et al., 2003), 64 were marine fishes, of which 32 were sharks and rays. It is worth noting that exploitation was the cause of extinction for all 32 of the sharks and rays (Dulvy et al., 2003; Camhi et al., 2008).

Although there is a fairly large amount of published data on the reproductive biology of sharks, there have been few published studies on the biology of batoid rays (Dulvy et al., 2003; Dabruzzi et al., 2012). The importance of acquiring such data for the dasyatidae family, in particular, is enhanced by this family alone containing more than 60 species and for being the dominant batoid family on the continental shelves of tropical and subtropical regions of the world (White and Dharmadi, 2007).

The ribbontail stingray or bluespotted stingray, *Taeniura lymma* (Forsskål, 1775) is amongst the species recorded on various landing sites of the Indo-West Pacific (Stevens et al., 2000; White and Dharmadi, 2007; Unsworth and Cullen, 2010; Dabruzzi et al., 2012). They may be found in shallow continental shelf waters ranging from temperate

to tropical seas and prefer areas with sandy or sedimentary substrates in which they bury themselves (Taylor, 1997; Bonfil and Abdallah, 2004; Wilborn, 2007; Last and Stevens, 2009). Sightings of *T. lymma* have been recorded in Australia in shallow tropical marine waters from Ningaloo Reef, Western Australia to Bundaberg, Queensland. They can be found at depths of up to 25m and have also been recorded to range in location from southern Africa and the Red Sea to the Solomon Islands (Taylor, 1997; Bonfil and Abdallah, 2004).

T. lymma is a colourful stingray with distinct, large, bright blue spots on its oval body (Lieske and Myers, 1994; Taylor, 1997; Bonfil and Abdallah, 2004). As adults, they are olive-grey or grey-brown dorsally and white ventrally, whereas newborns are pale grey or brown with black or blue dots as body pattern, distinct to each individual within a litter. The snout is rounded and angular with broad outer corners. The tail extension can be equal or slightly less than the body length when intact (Bonfil and Abdallah, 2004). Its caudal fin is broad and reaches to the tip of the tail. At the end of the tail there are two sharp venomous spines that they use as defence (Last and Stevens, 2009). The tail of *T. lymma* can be distinguished by the blue side-stripes found on either side. It has large spiracles that lie very close to its eyes (Lieske and Myers, 1994; Taylor, 1997). It may grow to a total length of 70cm and an average disk width of 35cm but larger rays have been reported (Bonfil and Abdallah, 2004; Last and Stevens, 2009). The mouth is found on the underside of the body along with the gills. Within the mouth are two plates, which are used for crushing the shells of crabs, prawns, and molluscs (Bonfil and Abdallah, 2004). Adults and juvenile rays occupy dissimilar habitats (Dabruzzi et al., 2012). Adults are found in cool deeper reef environments whereas juveniles frequent warm, shallow intertidal seagrass, mangal or rocky shoreline habitats (O’Shea et al., 2012).

Ribbontail stingrays are also the apex predator in benthic habitats within their range, feeding on small bivalves, soft-bodied annelids, cephalopods and crustaceans (Bonfil and Abdallah, 2004; Last and Stevens, 2009) they expose by excavating deep pits in sandy areas. Consequently they play an important ecologic role as bioturbators (O'Shea, 2012), modelling benthic morphology as well as determining density and distribution patterns of benthic infauna (O'Shea et al., 2012).

Regarding reproduction, *T. lymma* is described as aplacental viviparous, with females giving birth to litters of up to seven young (Taylor, 1997; Last and Stevens, 2009; Mull et al., 2011). During early gestation the egg capsule ruptures and disappears, either being voided or resorbed. Early development of the embryos is nourished by yolk stored in the egg. The yolk is fully utilized by the midpoint of gestation and the yolk sac is resorbed. During the latter half of gestation, the wall of the female's uterus becomes modified with dense fingerlike projections called trophonemata that extend into the lumen of the uterus. Specialized secretory cells on the elongated trophonemata produce a rich nutritious fluid called histotroph or "uterine milk." The embryos are bathed in this fluid, which they either ingest or passively absorb as the primary source of their nutrition during the latter half of development (Camhi et al., 2008).

During courtship, the male often follows the female with his acutely sensitive nose close to her cloaca in search of a chemical signal that the female will emit. Courtship usually includes some biting of the female disc. The teeth of the male are used to hold the female in place during population. The male fertilizes the female via internal fertilization through the use of their claspers (Camhi et al., 2008). Literature suggests that males do not become sexually mature until they reach a disk width of 21cm (White and Dharmadi, 2007; Dabruzzi et al., 2012) and it is probable that females mature at a larger width (Gianeti, 2011). Gestation period can be from 4 months to a year making them more susceptible to population collapse (Taylor, 1997; White and Dharmadi, 2007).

This species decline from over-fishing and changing habitat conditions has prompted the International Union for Conservation of Nature and Natural Resources to classify this species as near threatened with an unknown population trend (IUCN, 2012). Even though it is quite common and widely distributed, this species is greatly affected by human actions, due to capture in intensive inshore fisheries, its attractiveness for the marine aquarium fish trade (because of the small size and brilliant colour body pattern) and mostly, by the widespread destruction of its reef habitat (Cavanagh et al., 2003). To face these alarming marine biodiversity depletion, effective, sustainable and multidisciplinary *in situ* and *ex situ* conservation measures must be considered and applied (Curio, 1996; Stevens et al., 2000; Mallinson, 2001; Fowler et al., 2002; Dulvy et al., 2008; Veitch et al., 2012). The importance of captive breeding programs is now

recognized and encouraged for reinforcing conservations efforts in the wild (Mallison, 2001; Mallinson, 2003; Janse et al., 2012).

Public Aquaria may play a very important role in conservation of threatened species with or without commercial value, such as elasmobranchs, through specific captive breeding programs (Penning et al., 2009; Janse et al., 2012). These institutions also have a major part in public awareness concerning conservation of biodiversity, through the message they convey to millions of daily visitors and education potential that this fact entails (Smale et al., 2004; Wilcox, 2008; Penning et al., 2009).

The availability of specimens belonging to several species, access to captive history, knowledge and control of environmental parameters are some advantages of captive elasmobranch research. There are also some limitations to be considered like small sample sizes, minimal comparability with wild conspecifics and even captive conspecifics kept in different institutions (Smale et al., 2004). In spite of the constraints, captive elasmobranchs can contribute valuable information on biology, disease and treatments, blood chemistry baselines and sensitivity to environmental influences such as pollutants and habitat change. Some of the species critically threatened by overfishing, habitat change or other causes can be bred, and data compiled that may contribute to their conservation (Fowler et al., 2002; Smale et al., 2004; Janse et al., 2012).

The European Studbook (ESB) is one of the breeding programs the European Association of Zoos and Aquaria (EAZA) developed. The studbook keeper coordinates it and collects all the data on births, deaths and transfers from all the EAZA zoos and aquariums that keep the species in question. These data are entered in special computer software programs, which allow the studbook keeper to carry out analyses of the population of that species and provide recommendations on breeding or transfers to other EAZA institutions. The first ESB was established in 1996 but it was only in 2007 than the first ESB was established for a marine species, the zebra shark *Stegostoma fasciatum* (Hermann, 1783) (Penning et al., 2009). *T. lymma* was considered an important species to control and monitor through a breeding program by the Fish and Aquatic Invertebrate Taxon Advisory Group (TAG) of EAZA and it became an official ESB after approval of the European Endangered species Programs (EEP) Committee in 2007 (Baylina, 2011). In April 2013, this need for a breeding program was reconfirmed

during a meeting of the Elasmobranchs working group under the umbrella of the TAG. The studbook is currently on its second edition with the participation of 25 institutions (Baylina, 2013). The *T. lymma* ESB goals are to compile general husbandry information and husbandry practices for captive breeding, to increase the numbers of captive born animals and its survival in order to keep a sustainable and well managed captive population and decrease the number of captures in the wild (Baylina, 2013).

This study aims to highlight and apply suitable conditions for captive breeding of *T. lymma* in Oceanário de Lisboa by the establishment of a reproductive group. It also intends to study juvenile growth through a specific training, feeding and data collection plan. The transfer of specimens to other EAZA institutions was also a goal to achieve. During this study, 15 *T. lymma* were born and successfully raised in Oceanário and by September 2013, 11 animals were transferred to other European aquariums.

2. Breeding and juvenile growth of the ribbontail stingray *Taeniura lymma* at Oceanário de Lisboa

2.1 Introduction

Chondrichthyans face an escalating fishing pressure at a global scale, due to a combination of rising consumer demand and life history characteristics, which make them particularly vulnerable to over-exploitation (Stevens et al., 2000; Dulvy et al., 2008; O'Shea, 2012). Skates and rays are a particular susceptible and poorly studied among elasmobranch species (Dulvy et al., 2000; White and Dharmadi, 2007; O'Shea et al., 2012). Urgent conservation measures are needed to assure this species sustainability in the future (Rogers and Laffoley, 2011).

Elasmobranchs are very popular in Public Aquariums and have been kept in captivity for several decades. It is now recognized the importance of creating sustainable breeding programs, independent from wild animals, especially when it concerns threatened or vulnerable species (Wood, 2001; Jones et al., 2004; Penning et al., 2009). The work developed in Public Aquaria is a significant contribution to the conservation of threatened species, with or without commercial value, such as elasmobranchs (Fowler et al., 2002; Reid, 2006; Janse et al., 2012). These institutions also have a major part in public awareness, concerning conservation of biodiversity, through the message they communicate to millions of daily visitors and education potential that this fact entails (Smale et al., 2004; Wilcox, 2008; Penning et al., 2009).

The majority of elasmobranch reproductive behaviors reported in the literature have been observed in captive specimens, as it is difficult to closely monitor wild conspecifics. One hundred species of chondrichthyans are known to have exhibited reproductive behaviors or reproduced in captivity (Henningsen et al., 2004).

A captive breeding program (European Studbook) was established for *Taeniura lymma* (Forsskål, 1775) in 2007, being one of only three marine species embraced by this program. By then, only two European institutions had successfully bred this species but only one had juveniles growing (Baylina, 2011). At the present, 25 institutions participate in this program coordinated by the Oceanário de Lisboa and six institutions

now hold breeding pairs (Baylina, 2013). This species is classified as "near-threatened" by the IUCN (2012), due mostly to the widespread destruction of its reef habitat (Cavanagh et al., 2003), intensive exploitation by commercial and artisanal fisheries and by its attractiveness to the marine aquarium fish trade (White and Dharmadi, 2007; Unsworth and Cullen, 2010; Dabruzzi et al., 2012). Being a chondrichthyan, its life-history strategy, characterized by slow growth, late attainment of sexual maturity, long life spans and low fecundity (Carrier et al., 2004; Camhi et al., 2008; Dulvy et al., 2008) only aggravates its vulnerability to those threats.

Considering the ecological and economic value of ribbontail stingrays as well as their decreasing numbers, a better knowledge of this species could be valuable to develop sustainable management strategies for this fish (Mistry et al., 2005; Dabruzzi et al., 2012). *T. lymma* has been kept in captivity for several years with modest success. Success in animal care and exhibition is commonly measured by breeding, longevity and observations that the animal is demonstrating normal or natural behaviours (Sabalones et al., 2004).

EAZA reported 112 specimens register in the ESB and the living population counts 54 rays distributed by 19 institutions. Although the captive birth numbers have risen over the last three years, the percentage of captive born animals in the current living population is 33% (Baylina, 2013). The main challenges for keeping this species in captivity have been related with the post-shipment and adaptation period to quarantine (Thomas, 2005), vulnerability to parasite infection (Jensen, 2006; Olson et al., 2010) and reduced feeding (specially in juveniles), resulting in situations of anorexia and in the most serious cases, animal death (Thomas, 2005; Wilcox, 2008; Baylina, 2013).

Elasmobranch research along with husbandry techniques in public aquaria has evolved considerably over the last decades (Mohan et al., 2004; Koob, 2004; Smale et al., 2004). Areas of study comply amongst other, Physiology, Pathology, Behaviour, Diet and Nutrition, Population Dynamics and Growth and Development (Smale et al., 2004). Public aquariums have the potential to study the growth and early life stages of rare elasmobranch species. There are some studies concerning elasmobranch growth but they are normally performed using wild specimens and estimated parameters for age, considering growth bands deposited in calcified structures like vertebral centrum (Kusher et al., 1992) that usually requires animals' lethal sampling (O'Shea, 2012).

Coupling length and weight data with nutrition information yields a powerful tool for the assessment of husbandry techniques, allowing an assessment of the adequacy of a given feeding regime (Smale et al., 2004). This is the only environment where precisely time repeated measures of individual animals can be accomplished (Mohan et al., 2004).

One of the main goals of aquariums is to present species, as they would be seen in nature, with as much natural behaviour as possible. Furthermore, for measuring the success of an animal's acclimatization to controlled environments, its continued display of natural behaviours is usually considered (Sabalones et al., 2004). Due to their highly developed vision, acute sense of smell, lateral line and electroreception systems, elasmobranchs have an enormous potential for learning and exhibit complex behaviours. In captivity, they quickly learn the location of feeding stations and the timing of regular feeding sessions (Sabalones et al., 2004).

Animal training, through the use of operant conditioning, has been a key factor of the husbandry of marine mammals and has been applied over the last few years to birds, reptiles and fish (Sabalones et al., 2004; Corwin, 2012; Marranzino, 2013). These programs rely primarily on the positive reinforcement of successive approximations to a behavioural goal. Trained behaviour demands constant and continued reinforcement. It is therefore imperative that institutional commitment and support are ensured before a training program is implemented (Sabalones et al., 2004; Penning et al., 2009). Several benefits can be accomplished through the implementation of animal training programs, including: reduced stress to animals while handling and performing veterinary exams; more accurate control and monitoring of individuals and groups, during feeding sessions; enrichment, by physical and mental stimulation of trained animals; the establishment of advanced husbandry techniques and the improvement of educational presentations (Sabalones et al., 2004; Marranzino, 2013).

Literature discloses only a few studies performed with captive specimens of *T. lymma* (Rosemberger and Westneat, 1999; Thomas, 2005; Wilborn, 2007; Wilcox, 2008; Dabruzzi et al., 2012; O'Shea, 2012) but most of them (if not all) were performed with wild collected specimens.

This study might be the first analysis on juvenile growth of *T. lymma* bred and raised in captivity.

2.2 Materials and Methods

2.2.1 Establishment of a reproductive group

2.2.1.1 Animal collection, acclimatization and environmental parameters

Five *T. lymma* individuals were collected from the wild, transported and acclimated to Oceanário de Lisboa (ODL) facilities. The four females (TL1, TL2, TL3 and TL4) were collected in 2007 and the male (TL5), later in 2009.

After a short period in quarantine, the rays were introduced in the Tropical Habitat exhibit, a $243m^3$ cubic (10m x 3,5m) tank. The aquarium decoration consisted of artificial corals and the substrate was coral sand. *T. lymma* individuals inhabited this aquarium with more than fifty species of tropical fish. There were no potential predator species for *T. lymma* present (detailed information about this aquarium characteristics can be consulted in appendix 1).

Water quality parameters were defined according to the species needs and previous experience of Oceanário's aquarists in keeping this species. Parameters range is described in Table I. Water samples were collected and analysed on a daily basis and all maintenance actions: water changes, cleaning and Life Support System (LSS) adjustments were performed in order to keep the established parameters (the parameters frequency of analysis and the methods used are indicated in the appendix 2).

The reproductive group was held over natural lighting conditions (approximately 12:12h L: D) being supplemented with artificial light on overcast days.

Table I – Water quality parameters established to keep *T. lymma* specimens.

Parameter	Range
Temperature	24.5- 25.5°C
Salinity	32.5 – 33.5ppt
pH	8.15 – 8.25
Dissolved Oxygen	6.7-7.1mg.L^{-1}/ 96-100%
Ammonia	0.000-0.002mg.L^{-1}
Nitrite	0.002-0.003mg.L^{-1}
Nitrate	10-13mg.L^{-1}
Photoperiod	8-9h light

2.2.1.2 Monitoring, training and biometric data collection

The code "TL" followed by a number, from one to five identified the rays. They were distinguished by their individual blue dot body pattern and sexual dimorphism. Animals were monitored continuously, with daily observations and a specific training plan was applied in order to feed them and follow their growth individually.

The training plan included two phases. Phase one, where the animals were desensitized to target feeding sessions performed by a diver on four different levels of depth, starting on the place the animals were in the aquarium, progressing to the central bottom of the exhibit until reaching a shallow area (2m^2 and 25cm deep), chosen as the final feeding station. An acrylic platform (appendix 3) was used as substrate for the training steps performed between the bottom of the aquarium and the final feeding station.

Phase two began after the previous was successfully accomplished and regarded biometric data collection and veterinary exams. Target feeding sessions were performed from the surface from this point on.

A time line of 1-2 weeks was predicted for each step of the training to be accomplished and training success was measured by the positive response of each animal to perform it and to achieve the training goals.

During feeding sessions, the target (appendix 3) was presented to each ray and food was hand given as reinforcement to progressive approximations to the target, until it was only given when the rays actively pursuit the target and touch it in order to be fed.

The rays were fed six days a week, one time per day with 3% body weight. Food items included grass shrimp, peeled shrimp, clams, mussels, squid and various types of fish (capelin, sprat, herring and epperlan) chopped in pieces according to the mouth size of the animals. A vitamin supplement was provided once a week, consisting on a multivitamin pellet for elasmobranchs, potassium iodine and vitamin C, prescribed by the ODL veterinary and acquired from Premix ©.

Monitoring was continuously performed until the rays became sexually mature and therefor capable of breeding.

Pregnant females were identified at first visually and each pregnancy was always confirmed by an ultrasound exam. The pregnant females were monitored carefully until a later stage of the gestation, when they were transferred to quarantine in order to give birth. Ultrasound exams were performed regularly throughout each gestation.

Biometric data of the breeding group was collected on phase two of the training plan. The rays were monthly weighted to the nearest gram and their disc width was measured every two months. Veterinary exams were performed accordingly.

Disc width (DW, cm) was measured using a measuring tape (±0,1cm), during the feeding sessions in the shallow area. Total body weight (g) was monthly recorded for each individual. Weighting sessions were performed capturing the rays in the shallow area with a stretcher and suspending it on a dynamometer (Euripesa©, DHS30Kg ±0,15% maximum capacity), above the water for five to ten seconds in order to obtain a stable weight value to the nearest gram. All biometric data collected from the breeding group was kept to record and for further comparison between individuals.

2.2.2 Husbandry of the juvenile rays

The majority of *T. lymma* pups were born in quarantine. The ones born in the exhibit aquarium were transferred to quarantine the same day.

The quarantine aquariums were $4.18m^3$ rectangular tanks, with a small square acrylic window. Half of the tanks was kept darker, with black plastics covering the lids and artificial decoration was added.

Water quality parameters established for quarantine aquariums were the same defined to the adults (Table I). Water samples were collected and analysed on a daily basis and all maintenance actions (water changes, cleaning and LSS adjustments) were performed in order to keep the established parameters.

Photoperiod was set to 8-9h of artificial light (two 1.60m fluorescent lamps, 58W, Philips©) and some natural light was also present, as the aquariums were located near a glass front of the building oriented west.

2.2.2.1 Feeding plan and biometric data collection

After birth, juvenile rays were identified by the code TL followed by an individual number and records were kept regarding sex, parenthood and specific body pattern characteristics. All juveniles were fed *ad libitum*. A feeding and weighting frequency plan was applied from the first day.

Juvenile rays were fed seven times a day (every two hours from 8:00h to 20:00h) from day 2 to 5, with food items being mysis shrimp and krill.

From day 5 to 3 months after birth, feeding frequency remained the same but new food items were added (ditch shrimp, small pieces of epperlan, capelin, herring, clams, peeled shrimp and mussels). During this period ditch shrimp and krill were always offered as the first meal.

Between 3 and 5 months, feeding frequency decreased to five times a day (8:00 and 20:00h meals were cut off). Epperlan, capelin, herring, squid, clams, peeled shrimp and mussels were the food items provided, chopped in pieces according to the mouth size of the rays. Juveniles were also target-fed.

All food items used for the adults and juveniles were acquired and kept frozen. They were defrosted everyday before being administrated.

Biometric data was collected from the day they were born. Individuals were caught using a rubber mesh and the same method used for the adults was applied to weigh and measure them. Disc width (DW, cm) was registered at birth and every other month for record proposes.

Weighting frequency was adjusted according to the individuals' development. In the first 2 weeks, pups were weighted every two days and then, twice a week until they reach 2 months. From then on, weighting sessions were perform once a week. Weighing frequency was increased if the animals were losing weight or showed signs of low appetite. Tube feeding procedures were applied if a ray stopped eating, lost weight or showed signs of anorexia. A ray would be tube fed if its weight dropped below 100g.

Within a minimum of 101 and a maximum of 416 days, healthy juvenile rays were transferred to other institutions. Some of the animals were tagged with PIT-Tags (Micro ID 1.4x8mm) before being relocated.

2.2.3 Statistical analysis

Independent samples t tests were used to test the null hypothesis that there were no significant differences between birth weight and birth disc width according to sex (males and females) and litter size (singles and twins).

A least squares regression was applied to fit the relationship between juveniles' body weight and days after birth, to test if the growth in weight of juvenile rays under the conditions presented, was linear at an early stage. Furthermore, a growth in weight daily rate was estimated.

The juveniles' body weight increase, measured at 30, 60 and 90 days after birth was tested using repeated measures analysis of variance (ANOVA), considering sex and litter size as factors.

The assumptions of different statistic tests were previously tested. Significant differences were considered for p values <0.05.

Statistical analyses were performed using IBM SPSS Statistics v.22.0 (IBM Corp. ©**).**

2.3 Results

2.3.1 Training, breeding and gestation

Training included five steps and predicted a maximum of two weeks for a ray to consistently show the desired behaviour on each step, thus a minimum of twelve weeks was expected for each animal to complete training.

The training goals were successfully achieved for all 5 rays but their times of response were clearly different. The maximum time spent to complete training was 36 weeks for TL1 and TL2 and the minimum was eight weeks for TL5. TL3 and TL4 showed an intermediate response and took 28 weeks to complete all training steps in a consistent way.

The first training step regarding target feeding was accomplished for each ray in less than one week while the fourth and last steps, that demanded the rays to come to a platform placed close to the surface and swim into the shallow area to feed respectively, were the most difficult ones to achieve.

Courtship behaviour was observed from late 2010, on which all rays had apparently achieved adult size: DW= 30cm for TL1, 33.5cm for TL2, 26.5cm for TL3, 28cm for TL4 and 28.5cm for TL5 (data from October the 7^{th}, 2010).

With the exception of TL2, all females bred more than once during this study and a maximum of three cycles per year were recorded (TL3).

Each pregnancy was detected visually, by an increase on the females' body volume and appetite and confirmed shortly after, with an ultrasound exam. Since an early stage of conception, this procedure allowed to check the embryos vital signs and perceive the litter size, while monitoring their development throughout gestation.

There were only two unsuccessful gestations, from TL3. On the first one, the fetus was found death after being delivered (TL17) and on the second one, two fetuses were removed by the induction of an abortion under anaesthesia.

Gestation period was assessed from the second pregnancy (for each female) and was estimated from the day the female was exposed to the male until the date of birth. Only successful pregnancies were considered for gestation period estimation. Gestations (n=7) ranged from 128 to 203 days with an average of 159 ± 27 days.

2.3.2 Births, husbandry and juvenile growth

From March 2011 to June 2013, a total of fifteen pups were born at Oceanário from ten successful gestations. Births occurred throughout the years in no specific season.

The maximum litter size was 2 pups and the sex ratio was 1.14 (M:F). The new-borns were considered single (s) or twins (t) regarding litter size. Table II illustrates individuals weight and size at birth, sex, parenthood, month of birth and the classification of single or twins.

The mean size and weight for all neonates were 14.3 ± 2.8cm DW and 194.9 ± 62.4g, respectively.

Table II – Captive born *T. lymma* at ODL, from March 2011 to June 2013. Individual identification code "TL"; sex: male (m) or female (f); birth weight (g); birth disc width (DW, cm); birth month and year; parenthood: mother (+TL5, the only male in the group) and classification of single (s) or twins (t).

Individual	Sex (m/f)	Birth Weight (g)	Birth DW (cm)	Birth Month (year)	Parenthood (+TL5)	Single (s)/ Twins (t)
TL6	m	175	9	March (2011)	TL3	s
TL7	f	200	12	August (2011)	TL3	s
TL8	f	114	12.5	December (2011)	TL3	t
TL9	f	106	11.5	December (2011)	TL3	t
TL10	m	320	19	December (2011)	TL4	s
TL11	m	140	13	May (2012)	TL1	s
TL12	m	192	15	July 2012)	TL4	t
TL13	f	260	17	July 2012)	TL4	t
TL14	f	252	15	October (2012)	TL1	s
TL15	m	237	18.5	January (2013)	TL4	t
TL16	f	274	18	January (2013)	TL4	t
TL18	m	157	14	April (2013)	TL1	t
TL19	m	136	13	April (2013)	TL1	t
TL20	f	175	13	June (2013)	TL3	t
TL21	m	185	14	June (2013)	TL3	t

There were no significant differences between the birth weight and the sex of the rays ($t= -0.135$, $p> 0.80$) or between birth weight and litter size (single and twins) ($t= 0.989$, $p> 0.30$). Similar results were obtained when comparing the birth disc width with sex ($t= 0.195$, $p> 0.80$) and birth disc width with litter size ($t= -0.666$, $p> 0.50$).

The juvenile rays were kept according to the previously defined water quality parameters (table I) and the mean values for their first 90 days of age are illustrated on table III. Photoperiod was kept stable on ≈ 8:45h/ 15:15h light/ dark.

Table III – Average environmental parameters (temperature, salinity, dissolved oxygen, pH, ammonia, nitrites, nitrates and photoperiod) on which *T. lymma* were kept in their first 90 days of life.

Parameter	Unit	Average (±SD)
Temperature	°C	24.8 (±0.4)
Salinity	ppt	33.1 (±0.3)
Dissolved oxygen	$mg.L^{-1}$	6.9 (±0.2)
	%	100 (±3)
pH		8.19 (±0.05)
Ammonia	$mg.L^{-1}$	0.002 (±0.015)
Nitrites	$mg.L^{-1}$	0.021 (±0.013)
Nitrates	$mg.L^{-1}$	10.1 (±3.0)
Photoperiod	hours of light	8:45 (±0:00)

As the rays were fed *ad libitum* and several times per day, a rigorous control over water quality parameters was done. Aquarium cleaning and water changes were necessary more than once a day, in order to maintain the established parameters and to keep only fresh food available to the animals. The significant amount of food administrated, aggravated the tendency for the pH to drop and the nitrites levels to rise. Daily 2-4% water changes and the regular change of filter cartridges maintained water quality parameters and only occasionally larger water changes were required.

Feeding was performed seven times per day from day 2 to 5 after birth. All juveniles started to feed during this period, even tough some rays took more time to feed consistently on a daily basis. The rays responded positively to target feeding sessions within a week, allowing the evaluation of their feeding behaviour and food ingestion. Weighting sessions were also effectively performed from the first days and resulted in a key factor to detect any decline in the juveniles' condition, concerning weight loss.

No live food was used and mysis shrimp and krill were successfully administrated as the rays' first food. The juveniles gradually accepted new food items, offered as they grew. Food preferences seemed to vary among individuals and with age, with younger rays choosing ditch shrimp or clams, while older individuals apparently favoured fish (sprat and herring) or squid. As food selection diverged frequently among individuals, the variety of food items offered, resulted in a suitable way to keep the rays interested

during feeding sessions, where they were frequently observed choosing their preferred food item over others.

This selective feeding behaviour had been observed before, during the establishment of the reproductive group. Young rays appeared to preferred shrimp at some point and didn't prey on chopped sprat or capelin, but these items became their preference as they matured to breeding adults.

During the first 10 to 15 days, while adapting to feeding, half of the rays lost weight. In one severe case, TL9 dropped weight to 85g within 20 days after birth. This meant over 20% body weight loss and the ray became emaciated and unresponsive to any attempt to feeding. A more intervenient approach was done, performing assisted tube feeding every 48h with 2-4ml of blended mysis, herring and clams, until it started to feed on it's own and recovered weight. During this period (from day 11 to 30 after birth) TL9 was weighted every day until its weigh established beyond 100g. No anesthetics were use during tube feeding sessions.

Overall, an increasing growth in weight was recorded for the juveniles raised at ODL facilities (figure 1).

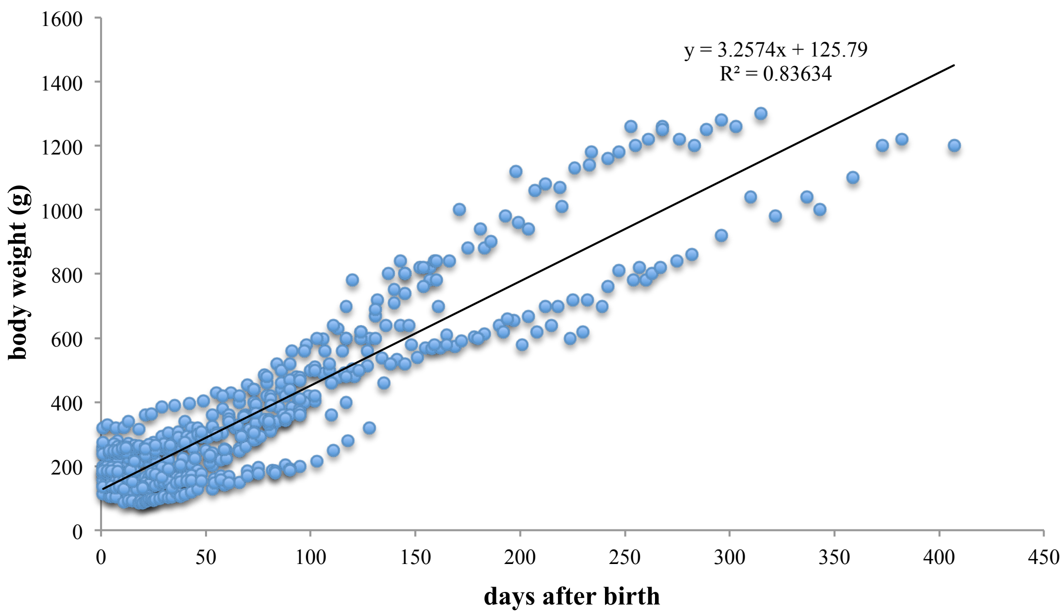

Figure 1 – Growth in weight (g) of the captive born *T. lymma* (n=15) from birth to a maximum of 416 days.

A lest squares regression fitted well to data recorded and showed that a juvenile ray, under the conditions of this study, may grow in weight in a 3.25g.day^{-1}rate. From birth to 150 days of age, all individuals showed a very similar growth in weight pattern but from 150 to 300 days, it is possible to distinguish two different groups, one growing over 1000g and the other staying below that value, taking more that 330 days to achieve that weight.

There were significant differences between individuals body weight increase measured at 30, 60 and 90 days after birth ($F= 71.84$, $p< 0.05$) however, those differences could not be associated with sex or litter. No significant differences were found when comparing weight increase over time between males and females ($F= 0.025$, $p> 0.80$) and between singles and twins ($F= 0.817$, $p> 0.30$).

2.4 Discussion

Oceanário de Lisboa kept a breeding group of *T. lymma* from 2001 to 2006, being one of the only two institutions in Europe with reported births for this species before 2007. However, the births were sporadic and the pups did not survive more than a few weeks. The initial group was lost due to some problems in the exhibit tank. Based on the previous reproductive success, it was decided to acquire a new group of rays to form a breeding group. Approximately one year later, the creation of the European Studbook coordinated by Oceanário de Lisboa reinforced this objective.

When provided the appropriate conditions, it is possible to successfully breed *T. lymma* in captivity. It is essential to gather a significant number of individuals and to monitor their welfare closely.

To monitor these animals individually seems to be an essential first step to take, to achieve the goal of successfully keep them in captivity and increase the chances of reproduction.

The method used to distinguish the rays, trough their body pattern is viable but can get difficult to ensure when we are dealing with a large group of individuals or while

observing or operating then within a distance (Wilcox, 2008). Some alternative methods can be used, like tagging with T-bars or PTI-tags (O'Shea, 2012).

The training plan contained several steps, was time consuming and required considerable logistics to be continuously performed. The six steps (setting goals, planning, implementation, documenting results, evaluating results regularly and re-adjusting) recommended by the American Zoo and Aquarium Association (AZA) (Sabalones et al., 2004) were followed. The approximations or steps defined were kept reasonable not to be too large that would frustrate the animals and trainers or too small that would waste time.

It was expected to obtain different responses to training between rays, regarding the time until a specific behaviour was consistently displayed, because each individual has its intrinsic characteristics. The faster responses of TL3, TL4 and TL5 are probably due to the fact that these rays had previously been subjected to target feeding sessions in quarantine. Furthermore, an animal later introduced in an exhibit to join a group of conspecifics, may learn from them (Sabalones et al., 2004). This might explain why the latter ray introduced in the group (TL5) was the fastest animal to accomplish all training steps.

The target presented to the rays was used as a visual and auditory cue (or discriminative stimulus) to invoke a specific behaviour, in this case, to touch it and follow it in order to be rewarded. The acrylic platform, later introduced as a substrate, was also a good prop for training progress. Being bottom dwellers it would be unnatural for these rays to feed from the water column. Once the mouth is located on the underside of the body, the rays press the prey into the substrate with their discs to trap food. The food is then conducted into the mouth by manoeuvring the disc over the prey (Taylor, 1997). Using a removable substrate, while the rays were progressively being led to the final feeding station, allowed them to keep exhibiting their natural feeding behaviour. The absence of this substrate might have hindered training (if the rays didn't feed from the water column) or have been misleading (having the animals to approach the diver for food and not going voluntarily to a certain place to be fed).

One way of lessening stress on multi species exhibits, is minimizing the investment of a species to consume its daily ration, without excess competition with their cohabitants using species-specific feeding stations (Charbeneu, 2004).

The final feeding station for the rays, a shallow 2m^2 area in the exhibit aquarium was chosen for being an accessible area, where competition for food from other species and conspecifics, was supervised and reduced (it was 25cm deep so other fish didn't entered easily). The shallow had also, fine river sand as substrate, providing a sheltered space where rays could excavate the substract in search for food, as they have been reported to do in the wild (Taylor, 1997; O'Shea, 2012). Finally, this shallow area was placed near the exhibit surface, thus being exposed to aquarium visitors, promoting public education and its awareness towards this species conservation. Curious visitors were frequently clarified about the work that was being performed and its purposes.

Feeding these rays in captivity has always been a challenge for many aquariums and considered as one of the biggest obstacles in their husbandry (Thomas, 2005; Wilcox, 2008; Baylina, 2011). As they appear to be selective feeders, both adults and juveniles, a varied set of food items, supplemented with vitamins, should be offered in every feeding, from early stages to adult age.

Vitamins are often administrated as supplements, to balance the loss of amino acids and other compounds due to pre-frozen and stored food, usually offered in aquariums. Examples of vitamins and minerals supplied are: vitamin C, which have a role in cartilage synthesis, works as antioxidant and is involved in maturation of erythrocytes; Potassium, a primary monovalent cation of intracellular fluid, involved in nerve reaction and osmoregulation and iodine, a constituent of thyroid hormone, frequently used to prevent goitre (Janse et al., 2004).

There are several brands in the current market, offering special vitamin supplements designed to fit elasmobranch requirements.

Wilcox (2008) analysed food preferences of captive *T. lymma* and peeler crab was identified as the preferred item even though the reasons for that preference still remain unclear. The author refers that for wild caught animals kept in captivity, food choices are normally related with their natural diet elements. For captive born animals, those choices, especially the ones that select items undescribed for the animal's natural diet, are yet to be discovered. Future food preferences studies would be useful to fulfil this lack of knowledge.

The most suitable diet for elasmobranchs in captivity has been defined as a copy of their natural diet (Janse et al., 2004). Often, this diet is undefined in literature (Janse et al., 2004; Wilcox, 2008). However, some studies have described the natural diets for elasmobranchs, with sharks been more frequently investigated than of rays. White et al. (2004), analysed the diet of *Rhynobatus typus* (Bennet, 1830) and three species of sharks from a subtropical embayment, finding that *R. typus* feeds almost exclusively on penaeid prawns. Recently, O'Shea (2012) studied the stomach contents of *T. lymma* in the Ningaloo Reef, Australia, where annelids were found to be the most dominant prey item. The author couldn't analyse stomach content for juveniles all stomachs of his samples were found empty.

Janse et al. (2004) compiled previous published information from different authors to suggest food elements for elasmobranch families kept in captivity. For Dasyatidae, the elements that should be part of their diet are crustaceans, teleosts, bivalves, polychaetes and cephalopods.

Neonates at Oceanário de Lisboa, accepted mysis shrimp and krill as first feed, revealing that live food is not an essential requirement for raising them. This is a very important aspect for the replication of this program in other institutions.

Feeding ration is also a subject of discussion among institutions keeping elasmobranchs in captivity. As feeding ration will depend on the type of food available, the age of the animal, health and hormone cycle, size and shape of the tank, water temperature and quality (Janse et al., 2004), each institution should evaluate a suitable feeding ration for the animals at its care. Generally, a 3-5% body weight per week is considered suitable and it is commonly used for adult sharks and rays (Carrier et al., 2004; Janse et al., 2004). Proper feeding, favorable environmental parameters and a healthy animal condition led to the reproduction of *T. lymma*, with a breeding group being successfully established, resulting in regular breeding events over the last 2 years.

From the biometric data collected it was possible to observe that courtship behavior and reproduction occurred when the male ray had grew over 28cm and the females over 26 to 33cm DW. These values concur with published data for maturity in this species, reported as 21cm DW for males and suggested slightly higher for females (White and Dharmadi, 2007; Gianeti, 2011). Pierce et al. (2009) reported minimum maturity sizes for *Neotrygon kuhlii* (Müller and Henle, 1841), a species often confused with *T. lymma*

for their similarities, as 28.7cm DW for males and 30cm for females, Other Dasyatidae species have been reported to mature at even larger sizes (Camhi et al., 2008; Henningsen and Leaf, 2010; Gianeti, 2011).

Courtship behavior and breeding occurred within no specific annual season and females showed a continuous reproductive cycle (maximum of three pregnancies in one year). This might be due to captive conditions being controlled thus, allowing dissimilar cycles than in a nature environment (Gianetti, 2011). An annual synchronous reproductive cycle has been reported for *N. kuhlii* (Pierce et al., 2009); a biannual cycle for *Pteroplatytrygon violacea* (Bonaparte, 1832) (Mollet et al., 2002) and also, a triannual cycle was described for the Mediterranean *Dasyatis Marmoratai* (Steindachner, 1892) (Canapé and Zaouali, 1995).

Considering that several species have been observed to mate immediately following parturition in captive elasmobranchs, whereas a longer gap is observed in the wild (Henningsen et al., 2004), this continuous breeding investment might have harmful consequences on the animals, mostly females. A break on the reproduction cycles, to allow females more time to recover from gestation and parturition, should be considered when facing this situation. During this study the female TL3 was observe to get pregnant less than 24h after parturition. Ideally to prevent consequent health problems, females should give birth in separate enclosures or be immediately removed from male exposure after parturition.

Previously published studies on *Dasyatis* rays have shown that their gestation periods vary from 3 to 11 months for those in subtropical waters being far shorter than those of temperate waters (White and Dharmadi, 2007), presumably because development time is strongly linked to water temperature (Wallman and Bennet, 2006).

The gestation period was estimated from the day a female was exposed to the male until the date of birth and could only be measured from each female second pregnancy, due to the exact day on which copula occurred could not be determined. It ranged from 128 to 203 days with an average of 159 ± 27 days. Similar results were described for captive *Dasyatis americana* (Hildebrand & Schroeder, 1928) (Henningsen, 2000), with gestation period ranging from 135 to 226 days. For wild *Dasyatis* species, 4 months gestations were published for *P. violacea* (Camhi et al., 2008), *N. kuhlii* (Pierce et al.,

2009) and for *Dasyatis guttata* (Bloch & Scneider, 1801) (Gianeti, 2011).

All females were monitored through the course of gestation using ultrasound exams to verify embryos vital sighs and the number of embryos present on each litter. The data collected from embryos during these exams will be part of another study in course. Ultrasounds have long been used as tools for diagnostic and reproduction studies in elasmobranchs (Walsh et al., 1993; Daly et al., 2007). It is a secure and non-invasive method to use in species like *T. lymma*, whose skin thickness is less disruptive to the propagation of ultrasound. It should be preferred over other high resolution but also more invasive methods like endoscopy, which might induce spontaneous abortions or premature births (Carrier et al., 2004).

Fifteen pups, eight males and seven females were successfully born and raised through the course of this study. The sex ratio was 1.14 (M:F). The sex ratio at birth has been equal and no different from 1:1 in *Dasyatis* species studied to date (Henningsen, 2000; Pierce et al., 2009; Henninsen and Leaf, 2010). No significant differences were found on birth weight and birth DW between males and females and between singles and twins. This might be real for *T. lymma* or a result of analysing a small sample. Henningsen (2000) studied a small captive population of *D. americana* and found no sexual dimorphism in the neonates DW but by enlarging his sample size, significant differences were found with females being larger at birth than males (Henningsen and Leaf, 2010). These findings are probably real because females tend to mature at larger sizes and grow to larger sizes, as described above.

The data for growth in weight of juveniles could be fitted with a linear regression and estimated a growth in weight rate of 3.25g per day. This agrees with the rapid growth rates described for juvenile elasmobranchs but a larger sample of *T. lymma* specimens must be analyzed to confirm this. Larger samples studies would also allow to explain the differences in growth at later stages of age and to estimate parameters to fit documented growth models for elasmobranchs. This would result in more accurate age and longevity estimates, which would highly contribute for a better management of the captive population. It is important to add, that juveniles might be vulnerable to weight loss in their initial live stages. In severe cases, tube feeding resulted in a positive method to stimulate their appetite while ensuring their nutritional needs (Thomas, 2005). This approach, in spite of being a more invasive procedure, has been used by

other institutions as a way to overcome situations where the animals were not feeding properly (Thomas 2005; Baylina, 2013).

Most fast growing elasmobranchs have relatively small maximum sizes and rapid growth rates last only one or two years as reported by Mohan et al. (2004) and Simpfendorfer et al. (2008). The latter author collected data for the small tooth sawfish indicating that growth from birth to one year of age resulted in an approximate doubling in size. Species growth rates at early stages of age are particularly important for aquarium management, especially when the aim is to provide captive specimens for other institutions. To know the time required for an animal to grow to a suitable size for transportation is essential for managing space availability within an aquarium infrastructure. Also, the number of individuals that an aquarium can keep and the actual species demand from other institutions must be taken into account when developing this kind of captive breeding programs.

Nutritional requirements are also important to know at different ages in order to maintain a proper and yet affordable feeding plan for the animals. Mollet et al. (2002) reported a decreasing food intake with age for *D. violace*a, with food consumption decreasing from 6-7% in the juvenile stage to 1.25% for adults. Although it has been suggested to simply increase the ration factor by 1.5-3.0% body weight per day (Janse et al., 2004) when feeding juveniles, future studies of food intake for *T. lymma* should be performed to progress from the *ad libitum* regime applied for juveniles to a less expendable feeding plan. Nevertheless, a high frequency feeding should be always performed in early stages of age for *T. lymma* specimens, to respond to their high metabolic rates.

From the 15 pups raised, 11 were successfully transferred to other institutions after 101 to 416 days. Three individuals were marked with pit-tags to facilitate their identification and future monitoring. A written summary with each individual characteristics and life history was also sent to the receiving institution.

Although growth in captive specimens may differ from wild conspecifics, studies of growth in laboratory and aquarium specimens have provided valuable life history information, particularly where none is otherwise available (Mollet et al., 2002; Mohan et al., 2004; O'Shea et al., 2012).

3. Final considerations and future studies

This research has identified key processes for the husbandry and captive breeding of the ribbontail stingray, *Taeniura lymma*. Individual monitoring of the rays and the appropriate environmental conditions, made possible to establish a successful breeding group and to obtain a significant number of juveniles.

For the first time, growth in weight has been quantified for juveniles, allowing biologists to understand this species requirements to develop and growth into healthy adults. The number of specimens a Public Aquarium can keep, and the time and space required to grow them, until they can be further transferred to other institutions is often limited, thus the knowledge of gestation periods and accurate weights and sizes at age are crucial for management.

This thesis also describes step-by-step, innovative husbandry techniques that can be applied in the future, by other institutions that have the interest and the conditions to breed *T. lymma*. The feeding plan implemented has demonstrated that is possible to raise this species without the use of live food, which could be an important constraint.

Despite the limitations of analysing small samples, this study provides useful information for future work to experimentally assess the biological impact environmental parameters have, on the reproduction and growth of these rays.

By raising the numbers of institutions breeding the ribbontail stingray it is possible to reduce the number of wild specimens capture every year for aquarium display. This study is one good example that captive breeding programs are important conservation strategies and once executed at a larger scale, might reduce the impacts on wild populations.

Future actions like obtaining DNA samples and biometric data of a wider group of specimens will provide knowledge for a better management of the captive populations and for further comparison with wild conspecifics.

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Appendices

Appendix 1: Exhibit and quarantine aquariums: Life Support System (LSS) and characteristics.

Exhibit – The LSS consisted of two pumps that propel the water from the bottom of the aquarium through a set of two sand filters at an average flow of $160m^3/h$. Then, the circuit was divided in two sets of pipes: one going into the heat-exchanger and through a set of U.V. filters and the other set, going directly to the U.V's. They join again and the water was propelled back into the tank. The water coming from the four surface skimmers went through a protein skimmer (at an average flow of 50m3/h), was treated with ozone injection, flowed by gravity into a degas tower and was pumped back into the aquarium. The water inflows through multiple pipes placed at half-depth and near the bottom creating water circulation along the aquarium.
The aquarium had 4 panoramic windows, 2 small detail windows and a $20m^2$ surface area visible from the top.

Quarantine – The quarantine aquariums were $4.18m^3$ rectangular tanks, with a small square acrylic window. LSS consisted of a set of eight cartridge filters (average of $11m^3$ water flow), four UV lamps and a biofilter tower. The aquariums had one bottom water outflow and two surface skimmers. The water returns to the aquarium by two inlets, one located near the bottom coming from the biotower and another one at the surface.

The tanks were covered with lids, consisting of a plastic black mesh (2cmx2cm) framed into PVC tubes. Half of each tank was kept dark (with black plastics covering the lids) and artificial decoration was added to create refugees for the rays and therefore, minimize stress levels during their stay in quarantine.

Appendix 2: Table 2a - Methods and frequency for water quality analyses.

Parameter	Method	Frequency
Temperature	Analog thermometer and Probe	Daily
Salinity	Conductivity	Every 48h
pH	Electrode	Daily
Dissolved Oxygen	Probe: Optical Method	1-2 x week
Ammonia	Colorimetric Method: Spectrophotometry	Daily
Nitrites	Colorimetric Method: Spectrophotometry	Daily
Nitrates	Colorimetric Method: Spectrophotometry	1- 2x week

Appendix 3: Instruments used in the training plan.

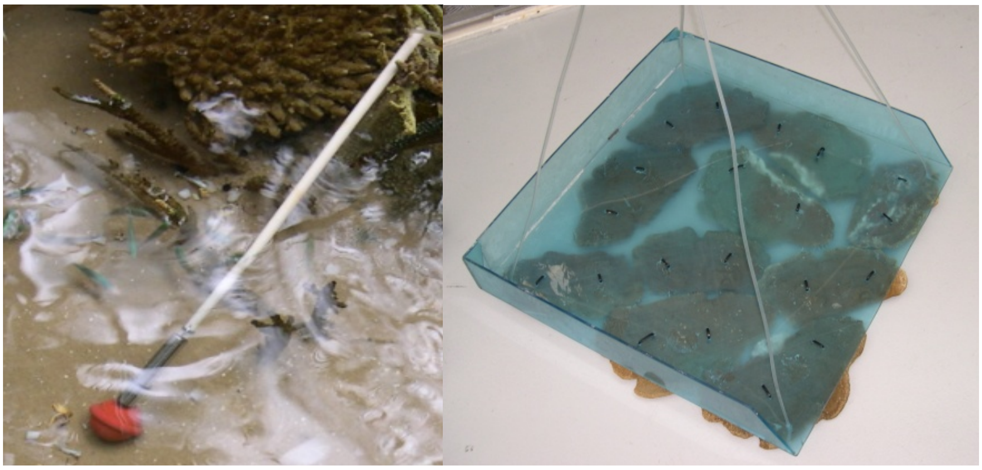

Figure 3a - Instruments used in the rays training. The target (left image) was built with a 30cm white PVC tube with an 8cm diameter red rubber ball placed at the end of the tube; the acrylic platform (right image) was made of a 5mm thick transparent blue acrylic. Plastic corals were attached to the bottom of the platform to break transparency. Dimensions were 50x40x15m.