Biology and Conservation

of

Freshwater Elasmobranchs

SYMPOSIUM PROCEEDINGS

R. Aidan Martin

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CONGRESS ACKNOWLEDGEMENTS

This volume is part of the Proceedings of the 6th International Congress on the Biology of Fish, held in Manaus, Brazil in August, 2004. Ten years have passed since the first meeting in this series was held in Vancouver, BC, Canada. Subsequent meetings were in San Francisco, California; Baltimore, Maryland; Aberdeen, Scotland; and again in Vancouver, Canada. From those meetings, colleagues from over 30 countries have contributed more than 2,500 papers to the Proceedings of over 80 Congress Symposia, all available for free viewing on the internet.

We would like to extend our sincere thanks to the many people who helped us organize the facilities and program for this 6th Congress.

The local arrangements team worked very hard to make this Congress a success. The leaders of those efforts were Vera Almeida Val, Adriana Chippari-Gomes, Nivia Pires Lopes and Maria de Nazare Paula Silva (Local Arrangements); Marcelo Perlingeiro (Executive Secretary) and Maria Angelica Laredo (Fund Raising). The enormous contribution of time and effort that was required has led to an unforgettable experience for the participants, thanks to the imagination, determination and dedication of this team.

Many sponsors helped ensure the success of the meeting through both monetary and in-kind contributions, including: Fundação Djalma Batista, Honda, Merse, Cometais, Turkys Aquarium, Banco da Amazônia, Banco do Brasil, FUCAPI, SEBRAE/AM, IDAM/SEPROR, FAPEAM, SECT-AM, SUFRAMA, PETROBRÁS, CAPES, FINEP, CNPq, the Physiology Section of the American Fisheries Society, UFAM - Federal University of Amazonas, Fisheries and Oceans Canada and INPA - National Institute for Research in the Amazon.

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The editorial team compiled the short abstracts into an abstract book and formatted and compiled the papers for the Symposium Proceedings. Thanks to Karin Howard, Christie MacKinlay, Anne Martin, Callan MacKinlay and Marcelo Perlingeiro.

In particular, we would like to extend a sincere ‘thank you’ to the organizers of the individual scientific Symposia and their many contributors who took the time to prepare a written submission for these proceedings. Their efforts are very much appreciated. We hope that their participation will result in new insights, new collaborations and new lines of research, leading to new papers to be presented at the 2006 Congress in St. John's, Newfoundland.

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Introduction

Freshwater elasmobranchs have been known for centuries but are not well known biologically. Their fishery management and conservation have received little study.

Salinity regimens used here are as follows:

Fresh water = salinity 0-10 ‰
Brackish = 11-32 ‰
Salt water = >‰

Following Compagno & Cook (1995a) freshwater elasmobranchs are divided into four habitat categories:

- Marginal (inshore marine, marginal in fresh water)
- Brackish Marginal (brackish to freshwater, marginal in rivers)
- Euryhaline (inshore marine, penetrating far up rivers into fresh water, far beyond tidal action; may breed in fresh water)
- Obligate Freshwater (occur only in fresh water)

Evolution

The earliest freshwater elasmobranchs were Xenacanthiformes, which arose in the upper Devonian (about 380 mya). Xenacanths appear to be the primitive sister taxon of the ctenacanths. Two families are recognized. *Orthacanthus*, from the Permian of Europe and North America, grew to a length of about 3 m. Xenacanths were widely distributed throughout what is now Europe, North America, and East Asia. This group persisted for nearly 200 million years, almost exclusively in freshwater habitats, until the end of the Triassic (about 215 mya).

In the Permian and Triassic, freshwater and brackish Hybodontiformes replaced the xenacanths. Hybodonts are derived protoselachians, sharing a common ancestor with xenacanths and ctenacanths. Six families are recognized. Hybodonts were widely distributed, found in freshwater and marine strata in what is now Europe, Spitzbergen, Greenland, North and South America, and southern Asia, and Australia. Freshwater hybodonts tended to be small. *Lissodus*, from the Permian of Africa (about 275 mya), was only 15 cm TL. Hybodonts persisted in freshwater habitats until the late Cretaceous (about 75 mya), surviving several million years longer than their marine relatives.

The earliest neoselachians to invade freshwater were probably the carcharhinoids, which arose during the late Jurassic (about 150 mya) in marine environments; it is not known when they first expanded to freshwater habitats. *Lamiopsis* is the primitive sister taxon to a clade formed by the euryhaline (*C. leucas* and *C. amboinensis*) + the euryhaline-obligate freshwater *Glyphis*.

Pristoids may have arisen as early as the late Cretaceous (about 100 mya), but the first undoubted pristid does not occur until the early Eocene (bout 57 mya). Pristids appear to have been euryhaline from their earliest appearance.

Myliobatoids arose in marine habitats of the late Cretaceous (about 100 mya). Some forms, e.g. *Heliobatis*, invaded fresh waters by the early Eocene (about 57 mya).

Potamotrygonids represent a monophyletic group derived from a Pacific *Urolophus*-like ancestor. Their common ancestor apparently became trapped in isolated freshwater habitats by orogenic events during the Paleocene-Miocene (65-23 mya). *Paratrygon* is the primitive sister taxon to the clade formed by

Plesiotrygon and *Potamotrygon*. The sister group to the potamotrygonids appears to be amphi-American *Himantura* (Thorson et al., 1983).

Diversity

Approximately 45 species of elasmobranch, in four families and ten genera, are found in fresh water far beyond tidal influences in rivers and estuaries; at least 48 additional elasmobranch species penetrate fresh water in estuaries or river mouths but are not found far from the sea. Diversity of freshwater elasmobranch is dominated by potamotrygonid and dasyatid stingrays, which together comprise almost half of freshwater elasmobranchs (Table 1). Extant obligate euryhaline and freshwater elasmobranchs comprise three relatively unspecialized ecomorphotypes (rajobenthic, pristobenthic, and littoral) and are largely restricted to tropical rivers and lakes. The low taxonomic, ecological, and morphological diversity of freshwater sharks and rays compared with freshwater bony fishes and marine cartilaginous fishes suggest that fresh water may be a marginal habitat for elasmobranchs.

Table 1: Summary of habitat distribution of freshwater elasmobranchs (Modified after and updated from Compagno & Cook 1995a)

1) MARGINAL SPECIES:

- Order Hexanchiformes
  - Cow Sharks – Family Hexanchidae
    - *Notorynchus* (1 species)
- Order Squaliformes
  - Spiny Dogfishes – Family Squalidae
    - *Squalus* (1 species)
  - Sleeper Sharks – Family Somniosidae
    - *Somniosus* (1 species)
- Order Lamniformes
  - Mackerel Sharks – Family Lamnidae
    - *Carcharodon* (1 species)
    - *Lamna* (1 species)
- Order Orectolobiformes
  - Long-Tailed Carpet Sharks – Family Hemiscylliidae
    - *Chiloscyllium* (1 species)
- Order Carcharhiniformes
  - Hound Sharks – Family Triakidae
    - *Mustelus* (2 species)
    - *Triakis* (1 species)
  - Requiem Sharks – Family Carcharhinidae
    - *Rhizoprionodon* (2 species)
    - *Scoliodon* (1 species)
    - *Carcharhinus* (6 species)
    - *Glyphis* (2 species)
    - *Negaprion* (1 species)
  - Hammerhead Sharks – Family Sphyrnidae
    - *Sphyrna* (2 species)
- Order Pristiformes
  - Sawfishes – Family Prisidae
    - *Pristis* (1 species)
- Order Rhiniformes
  - Wedgefishes – Family Rhinidae
    - *Rhynchobatus* (1 species)

Table 1: (continued)

1) MARGINAL SPECIES (cont'd): Order Rhinobatiformes Guitarfishes – Family Rhinobatidae *Rhinobatos* (2 species) Order Myliobatiformes Round Stingrays – Family Urolophidae *Urolophus* (1 species) Whiptail Stingrays – Family Dasyatidae *Dasyatis* (6 species) *Himantura* (2 species) (unidentified dasyatid, North Carolina) Butterfly Rays – Family Gymnuridae *Gymnura* (3 species) Eagle Rays – Family Myliobatidae *Aetobatus* (1 species) *Myliobatis* (2 species) Cownose Rays – Family Rhinopteridae *Rhinoptera* (2 species) 2) BRACKISH MARGINAL SPECIES Order Myliobatiformes Whiptail Stingrays – Family Dasyatidae *Dasyatis* (1 species) *Himantura* (1 species)	3) EURYHALINE SPECIES: Order Carcharhiniformes Requiem Sharks – Family Carcharhinidae *Carcharhinus* (1 species) *Glyphis* (3 species) Order Pristiformes Sawfishes – Family Pristidae *Anoxypristis* (1 species) *Pristis* (5 species) Order Myliobatiformes Whiptail Stingrays – Family Dasyatidae *Dasyatis* (2 species) *Himantura* (2 species) *Pastinachus* (1 species) 4) OBLIGATE FRESHWATER SPECIES: Order Carcharhiniformes Requiem Sharks – Family Carcharhinidae *Glyphis* (1 species) Order Myliobatiformes River Stingrays – Family Potamotrygonidae *Paratrygon* (1 species) *Plesiotrygon* (1 species) *Potamotrygon* (18 species) (undescribed potamotrygonid) Whiptail Stingrays – Family Dasyatidae *Dasyatis* (4 species) *Himantura* (4 species)

Taxonomic Problems

Taxonomic problems of fossil and extant freshwater elasmobranchs are summarized in Table 2.

Table 2: Taxonomic problems of fossil and extant freshwater elasmobranchs.

Taxon	Problem(s)
Antartilamna prisca	Does not appear to be a xenacanth
Aegyptobatis	Dubiously placed in Distobatidae
Asterocanthus eocaenus	Not a hybodont
Lissodus	Paraphyletic
Carcharhinus	Paraphyletic
Glyphis	3+ undescribed species
Pristidae	Systematics highly unsettled
Neotropical *Dasyatis*	Paraphyletic
Dasyatis ukpam	Does not appear to be a *Dasyatis*
Dasyatis sp. (China)	May be synonymous with *D. laosensis*
Himantura fluviatilis complex	3 species may be synonymous
Himantura krempfi	May be synonymous with *H. oxyrhyncha*
Potamotrygonidae	5+ undescribed species; many species inadequately defined; high degree of intraspecific polychromatism
Potamotrygon dumerilii and *P. humerosa*	Inadequately defined; lack material for proper characterization

Zoogeography

Some freshwater elasmobranchs occur in warm-temperate rivers such as the Mississippi River in the USA or the rivers of Natal in South Africa, but most occur in the tropics of both hemispheres.

The greatest diversity and endemism of freshwater elasmobranchs occurs in the Atlantic drainages of South America with its radiation of the Potamotrygonidae, but pockets of endemism and diversity also occur in West Africa and in Asia (from the Indian subcontinent eastward through Southeast Asia, southern China, Indonesia, New Guinea, the Philippines, and Australia). Freshwater elasmobranchs also occur in the Tigris River system of southern Iraq, from several rivers in Africa, North America, southern Europe (Portugal), and rivers draining into the Mediterranean Sea.

Zoogeography of representative euryhaline and freshwater elasmobranch taxa (*C. leucas*, *Glyphis* spp., pristids, *Dasyatis*, *Himantura*, and potamotrygonids) are summarized in Figures 1-6. Selected records of euryhaline and freshwater elasmobranchs more than 200 km upriver from the sea are presented in Table 3.

Table 3: selected records of euryhaline and freshwater elasmobranchs >200 km upriver. Data from Compagno and Cook (1995a).

Species	River	Distance from Sea (km)
Carcharhinus leucas	Mississippi	3800
	Amazon	4200
	Zambezi	1120
Pristis perotteti	Amazon	1340
Dasyatis sabina	Mississippi	322
D. ukpam	Old Calabar	241
Himantura fluviatilis	Ganges	1600
H. uarnak	Trembeling	354

Threats

The tropical rivers and lakes where most freshwater elasmobranchs occur are mostly in developing countries with enormous, rapidly expanding human populations. Increasing levels of direct exploitation and modification or destruction of riverine and lacustrine ecosystems – especially where uncontrolled human population growth is occurring – threaten many freshwater elasmobranch stocks and obligate freshwater species with extinction (Compagno and Cook, 1995b). Threats to freshwater elasmobranchs are summarized in Table 4.

Table 4: Threats to freshwater elasmobranchs

Threat	Mechanism(s)	Status
Fisheries	Targeted and untargeted (bycatch) removal; reduction of prey base; ornamental trade	Increasing
Deforestation	Increased microclimate modification; damage to soil; water siltation; flooding	On-going
Damming Rivers	Cut off access to sea; extreme conditions in reservoirs	Increasing
Mining	Introduction of heavy metal pollutants (Pb, Cu, Hg) & radioactive isotopes (U) toxic to elasmobranchs and their prey	Increasing
Illegal Drug Manufacturing	Introduction of organic chemicals toxic to elasmobranchs and their prey	On-going
Warfare	Introduction of petrochemical compounds & herbicides toxic to elasmobranchs and their prey; habitat modification via blasting & mining (see above)	On-going

Conservation

Freshwater elasmobranchs at greatest risk of human impact are obligate freshwater species with limited geographical distributions (such as many dasyatid and potamotrygonid stingrays and possibly the Ganges Shark) or euryhaline species trapped by man-made barriers that prevent free transit to estuaries and the ocean. Euryhaline elasmobranchs may be less vulnerable than obligate freshwater species, but are generally confined to warm inshore marine environments exploited via low-technology, increasingly intensive artisanal and small-scale commercial fisheries as well as tourist sports fisheries, and coastal development/degradation. Certain euryhaline elasmobranchs (*Pristis microdon*, *P. perotteti*, *Pastinachus sephen*, and possibly *Himantura fluviatilis*) reproduce in fresh water and are affected by anthropogenic problems in these areas (Compagno and Cook, 1995c; Góes de Arûjo et al., 2003).

Economic and political issues affecting freshwater elasmobranch conservation are summarized in Table 5. Priorities for research and management of freshwater elasmobranchs include, 1) better monitoring of tropical freshwater populations, 2) encouragement of elasmobranch conservationists in tropical countries, 3) fostering studies of their systematics, life history and ecology, and 4) development of management protocols.

Table 5: Economic and political issues affecting freshwater elasmobranchs

- Poverty
  - Hunger
  - Disease
  - Inadequate education
- Political Instability
  - Civil strife
  - Regional or civil wars
  - Corruption
  - Ineffective governance
- Tourism Development
  - Sport angling
  - Anti-shark measures
- Exploitation of New & Underutilized Stocks
  - Targeted exploitation
    - Food
    - Leather
    - Liver oil
    - Pharmaceuticals
    - Ornamental trade
      - Curios
      - Aquarium specimens
  - Bycatch

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Figure 1: Zoogeographic distribution of the euryhaline bull shark (*Carcharhinus leucas*)

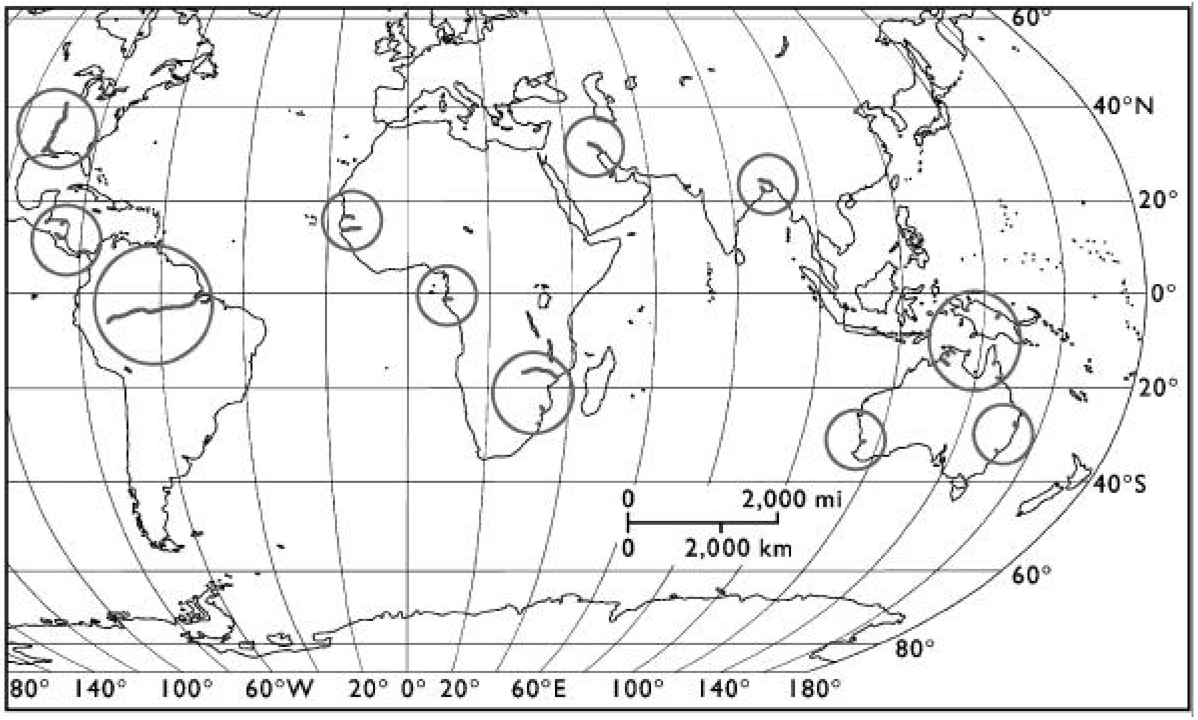


Figure 2: Zoogeographic distribution of the river sharks (*Glyphis* spp.)

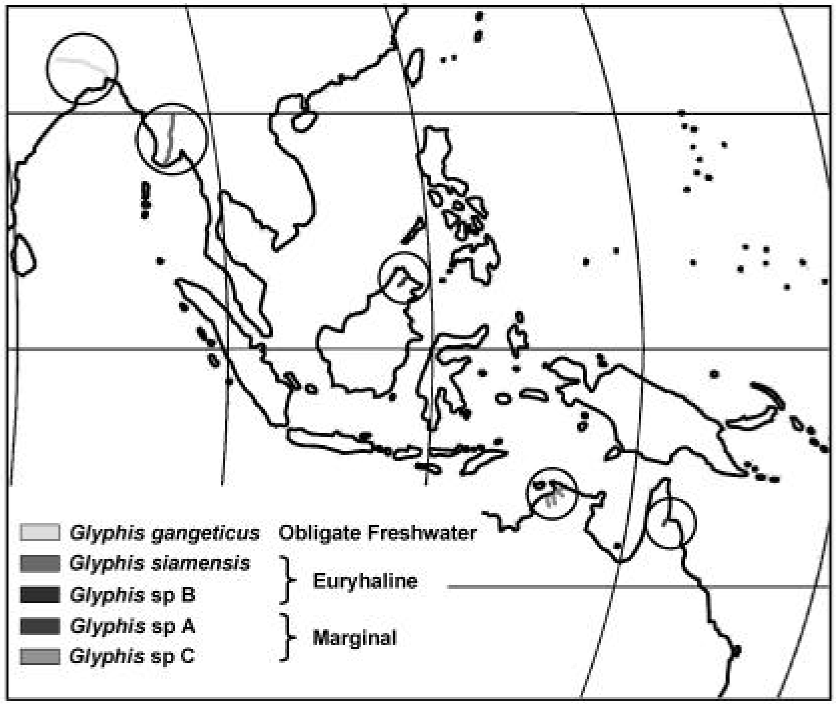


Figure 3: Zoogeographic distribution of the sawfishes (Pristidae)

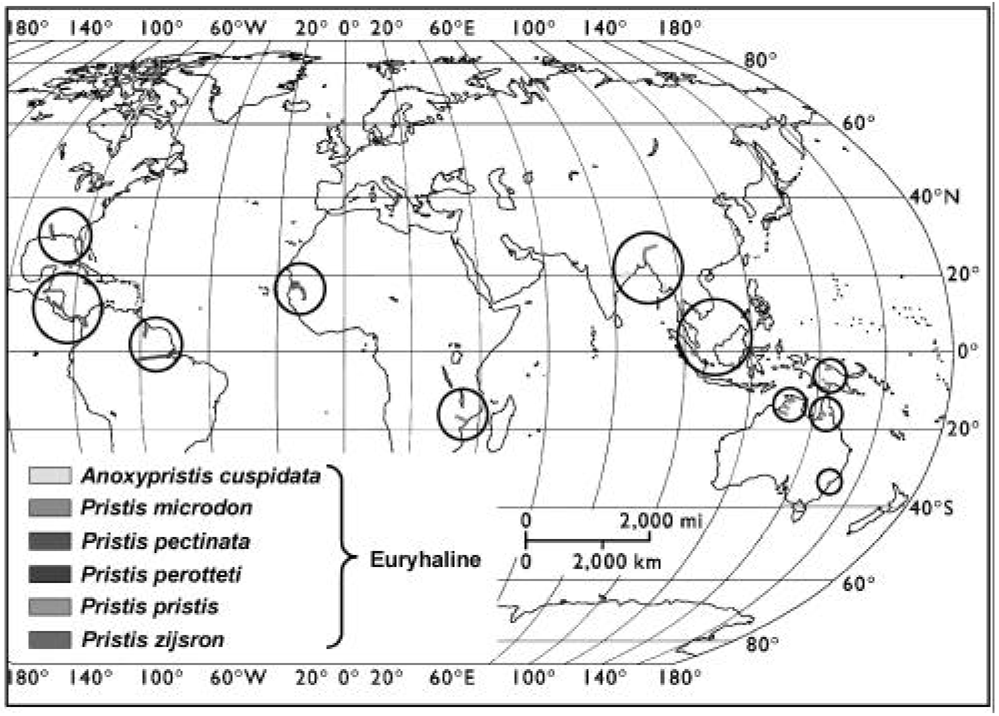


Figure 4: Zoogeographic distribution of fintail stingrays (*Dasyatis* spp.)

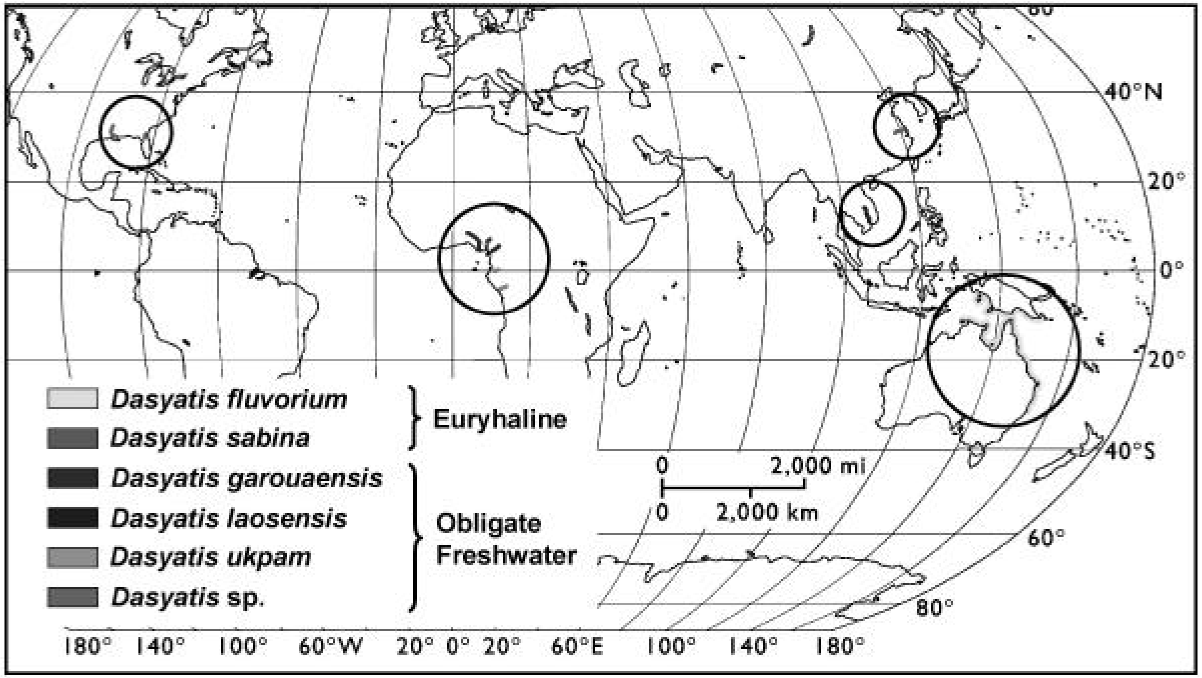


Figure 5: Zoogeographic distribution of the whip stingrays (*Himantura* spp.)

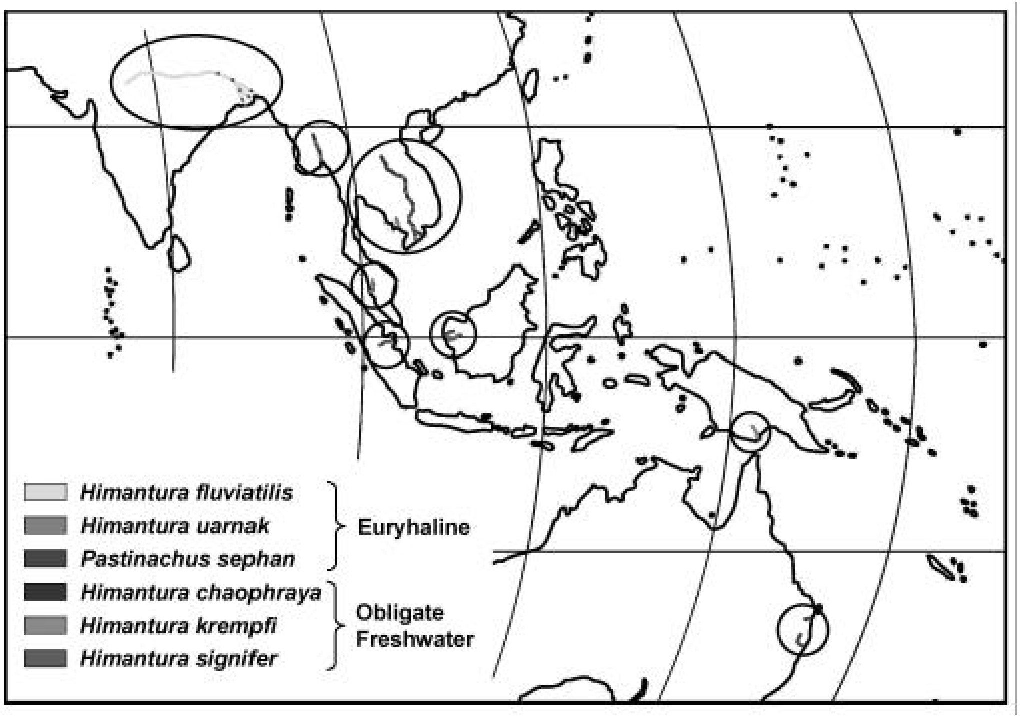
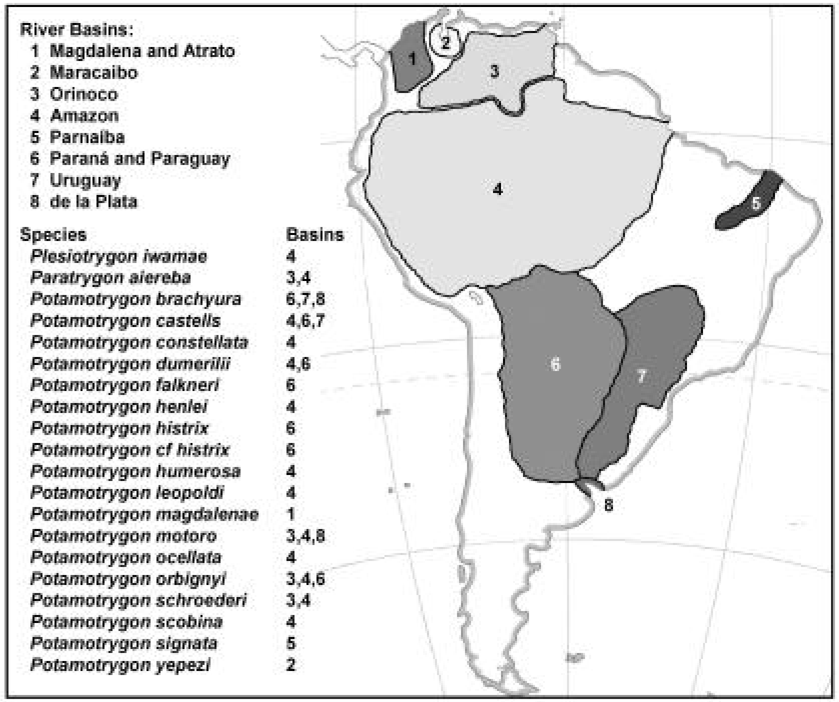


Figure 6: Zoogeographic distribution of the river stingrays (Potamotrygonidae)



River Basins:
1 Magdalena and Atrato
2 Maracaibo
3 Orinoco
4 Amazon
5 Parnaiba
6 Paraná and Paraguay
7 Uruguay
8 de la Plata

Species	Basins
Plesiotrygon iwamae	4
Paratrygon aiereba	3,4
Potamotrygon brachyura	6,7,8
Potamotrygon castells	4,6,7
Potamotrygon constellata	4
Potamotrygon dumerilii	4,6
Potamotrygon falkneri	6
Potamotrygon henlei	4
Potamotrygon histrix	6
Potamotrygon cf histrix	6
Potamotrygon humerosa	4
Potamotrygon leopoldi	4
Potamotrygon magdalenae	1
Potamotrygon motoro	3,4,8
Potamotrygon ocellata	4
Potamotrygon orbignyi	3,4,6
Potamotrygon schroederi	3,4
Potamotrygon scobina	4
Potamotrygon signata	5
Potamotrygon yepezi	2

FRESHWATER HYBODONT SHARKS FROM THE LOWER CRETACEOUS OF THAILAND

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Abstract

The lower Cretaceous of Thailand has yielded isolated teeth of eight hybodont genera, including *Hybodus, Lonchidion, Thaiodus* and *Heteroptychodus.* They are distributed in two successive assemblages, the first one from the Sao Khua Formation (Neocomian), and the second one from the Khok Kruat Formation (Aptian), with only two genera in common between these two assemblages, *Hybodus* and *Heteroptychodus*. These sharks show a wide range of diet and many of them were restricted to freshwater environment and thus endemic to the Khorat Plateau. The distribution of *Thaiodus* and *Heteroptychodus,* known in other Asian locations, may be explained by a mode of life similar to that of the modern sawfish *Pristis perotteti.*

Introduction

New data from the Early Cretaceous of Thailand show an unexpected diversity of hybodont sharks in freshwater environments, first in the Sao Khua Formation (Neocomian), and then in the Khok Kruat Formation (Aptian), with a total of 8 different genera recorded so far.

Geological settings

The Khorat Group is a set of sandstones, clays and freshwater limestones deposited during the Mesozoic in Northeastern Thailand (and parts of adjacent

Laos and Cambodia). It ranges in age from the Late Jurassic (Phu Kradung Formation) to the Cenomanian (Maha Sarakham Formation), and its total thickness is nearly 3200 m. Hybodont shark remains have been so far retrieved from the Phu Kradung Formation (Uppermost Jurassic), the Sao Khua Formation (Neocomian) and the Khok Kruat Formation (Aptian). This article will focus on the Sao Khua and Khok Kruat faunas.

Systematic description

Class: Chondrichthyes Huxley 1880
Subclass: Elasmobranchii Bonaparte 1838
Order: Hybodontiformes Maisey 1987
Family: Hybodontidae Owen 1846
Subfamily: Hybodontinae Maisey 1989
Genus: H*ybodus* Agassiz 1837
Hybodus sp. A

Occurences: Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation.

Description

Because of space restriction, the precise description of the teeth can be found in Cuny et al. (2003). See also Fig. 1A-C.

Discussion

The density of the ornamentation of these teeth is reminiscent to that of *Hybodus brevicostatus* from the Wealden of Britain (Patterson, 1966). However, the teeth from Thailand are easily distinguished from those of *H. brevicostatus* by the much better developed main cusp and lateral cusplets and the absence of lingual nodes at the base of the crown. These teeth also show an ornamentation quite similar to those of *Hybodus* sp. described from the Upper Jurassic of Ethiopia (Goodwin et al., 1999), but again are easily distinguished on the basis of the first pair of lateral cusplets almost as high as the main cusp and the absence of fine, short non-branching ridges on the cutting edge. The teeth from Thailand, with their first pair of lateral cusplets

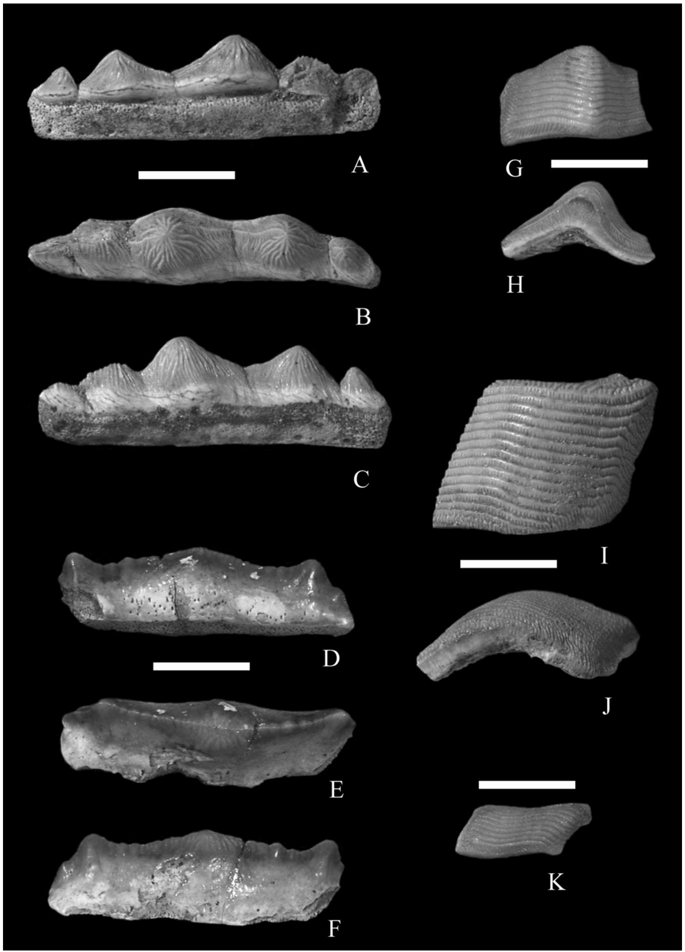

Fig. 1: A-C: Tooth (TF 7644) of *Hybodus* sp. A in A: labial, B: apical and C: lingual view. D-F: Tooth (TF 7646) of *Thaiodus ruchae* in D: labial, E: apical and F: lingual view. G-K: *Heteroptychodus steinmanni*. G-H: anterior tooth (TF 7647) in G: apical and H: labial view. I-J: lateral tooth (TF 7648) in I: apical and J: labial view. K: posterior tooth (TF 7649) in apical view. All scale bars: 5 mm. All the specimens come from Khok Pha Suam.

almost as high as the main cusp, appear to be fairly unique and probably belong to a new species.

Hybodus sp. B

Occurences: Phu Phan Thong, Nong Bua Lamphu province, Sao Khua Formation.

Description

See Cuny et al. (2003).

Family: Ptychodontidae Jaekel 1898
Genus: *Heteroptychodus* Yabe & Obata 1930
Heteroptychodus steinmanni Yabe & Obata 1930

Occurrences: Phu Phan Thong, Nong Bua Lamphu Province, Sao Khua Formation; Phu Wiang 1A, Khon Kaen Province, Sao Khua Formation; Phu Kum Khao, Kalasin Province, Sao Khua Formation; Phu Phok, Sakon Nakhon Province, Sao Khua Formation; Non Liam, Chaiya Phum Province, Sao Khua Formation; Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation.

Description

See Cuny et al. (2003) and fig. 1G-K and 2E-F.

Discussion

The dentition can be reconstructed as follows: Anterior teeth (TF 7647, fig. 1G, H) with a well developed bulge not in the centre of the crown, large crushing lateral teeth (TF 7648, fig. 1I, J) with a moderately developed bulge, and small, flattened posterior teeth (TF 7649, fig. 1K). Some teeth might belong to juveniles (Fig. 2E).

This genus was hitherto known only from a handful of teeth found in the Lower Cretaceous of Japan (Yabe & Obata, 1930; Tanimoto & Tanaka, 1998). However, hybodont teeth attributed to the genus *Asiadontus* were reported from the Aptian-Albian of Kirghisia and Mongolia (Nessov, 1997). The ornementation of these teeth is very similar to that of *Heteroptychodus* and it is likely that the two genera are synonyms. *Heteroptychodus* was thus present all around the Asian continent.

The teeth of *Heteroptychodus* differ from those of *Ptychodus* by a reduced marginal area and a denser pattern of parallel longitudinal ridges ornamenting the crown. The teeth with a well-developed bulge recall what is seen in *Ptychodus whipplei* (Williamson et al., 1993) or *P. rugosus* (Cappetta, 1987). The massive root, narrower than the crown, is also similar to that of *Ptychodus*. We therefore include *Heteroptychodus* in the family Ptychodontidae, which thus comprises two genera: *Heteroptychodus* and *Ptychodus*, as *Hylaeobatis* is now considered better included in the family Lonchidiidae (Batchelor & Ward, 1990; Rees & Underwood, 2002).

The teeth of the juveniles are more reminiscent of the ornamentation pattern of *Ptychodus* showing well-separated longitudinal ridges devoid of secondary ridges although the marginal area is still very reduced. The main difference however is the presence of one cusp on each of the ridges. However, if we compare with modern sharks possessing a grinding dentition, Reif (1976) has documented that the juveniles of the modern *Heterodontus* show a dentition that is not as grinding as that of the adult. It is therefore reasonable to think that this may have also been true of some Mesozoic hybodonts, although similar cusps, to the best of the knowledge of the authors, have never been reported in the genus *Ptychodus*. The presence of secondary ridges in *Heteroptychodus* appears to be size-related as labial ones are present only in the largest teeth. Their absence on juvenile teeth is therefore not surprising. The attribution of TF 7655 and similar teeth to a juvenile *Heteroptychodus*, which must be considered tentative, relies therefore on the hypothesis that their diet was different from that of the adults, and the fact that they share with the adult an ornamentation which is made of parallel longitudinal ridges with a reduced marginal area.

Family: *incertae sedis*
Genus: *Thaiodus* Cappetta, Buffetaut & Suteethorn 1990
Thaiodus ruchae Cappetta, Buffetaut & Suteethorn 1990

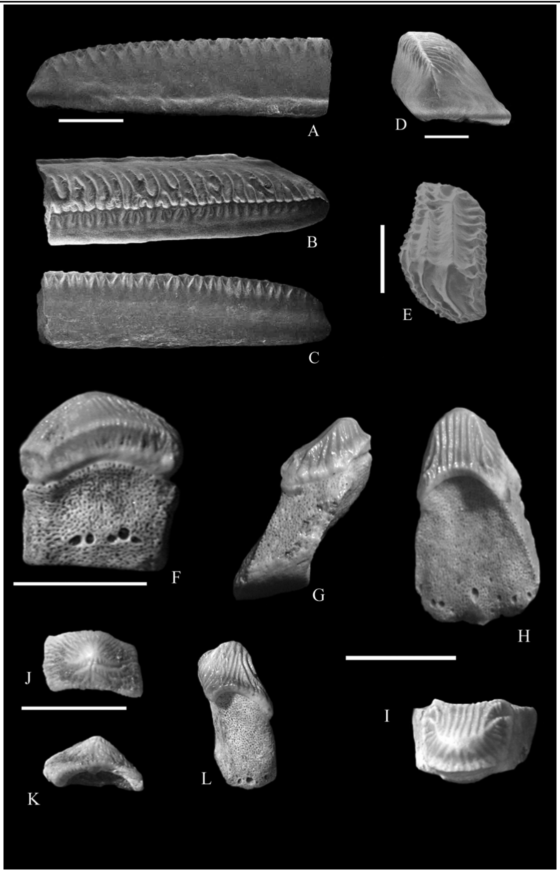

Fig. 2: A-D: Tooth (TF 7659) of genus #1 in A: lingual, B: apical, C: labial and D: mesial or distal view. E: tooth (TF 7655) of a juvenile *Heteroptychodus steinmanni* in apical view. F: Lateral tooth (TF 7650) of *Heteroptychodus steinmanni* in labial view. G-I: Tooth (TF 7657) of genus #2 in G: mesial or distal, H: lingual and I: apical view. J-L: Teeth of genus #3. J-K: TF 7651 in J: apical and K: lingual view. L: TF 7653 in lingual view. Scale bars: A-C, E: 1 mm, D: 0.5 mm, F-L: 5mm. All the specimens come from Khok Pha Suam.

Occurrences: Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation; Ban Khok Kruat, Nakhon Ratchasima Province, Khok Kruat Formation.

Description

See Cuny et al. (2003) and fig. 1D-F.

Discussion

The teeth of *Thaiodus ruchae,* currently known only from Thailand and Tibet (Cappetta et al., 1990) show a unique morphology characterized mainly by the presence of irregular, obtuse, serrated denticles and a highly asymmetric crown, the lingual face being concave and flared basally while the labial face is gently convex. However, new material currently under study by Cappetta might indicate that the orientation of the teeth used in this work is wrong, the labial face being the lingual one and vice versa. Anyway, these characters clearly separate these teeth from those of any Hybodontidae, even if one accepts the broad definition of this family by Maisey (1989), which includes the genera *Palaeobates, Asteracanthus, Bdellodus, Acrodus, Egertonodus* and *Hybodus,* plus *Tribodus* (Brito & Ferreira, 1989; Maisey & de Carvalho, 1997) and *Priohybodus* (Duffin, 2001). The inclusion of *Thaiodus ruchae* into the Hybodontidae by Cappetta et al. (1990), not justified by the possession of shared derived characters, seems therefore unlikely. *Thaiodus*, probably, belongs to a family of its own.

New genus and species #1

Occurrences: Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation

Description

See Cuny et al. (2003) and fig. 2A-D.

Discussion

The low crown profile and elongated shape of these crowns suggest that they formed crushing batteries, as in *Asteracanthus* (Rieppel, 1981) or *Acrodus* (Mutter, 1998). But unlike these two Hybodontidae, they share with the teeth of *Thaiodus ruchae* an asymmetric crown with a convex labial face and a basally flared lingual face. Moreover, both kinds of teeth share a mesio-distally elongated crown. This strongly suggests that these two genera are closely related.

New genus and species #2

Occurrences: Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation.

Description

See Cuny et al. (2003) and fig. 2G-I.

Discussion

These teeth share with *Thaiodus ruchae* and genus #1 a convex labial face and a concave, basally flared lingual face. They are easily distinguished from the two previous taxa by a strong ornamentation and teeth that are moderately elongated mesio-distally. According to the small number of teeth available and their rather poor preservation, the heterodonty of this species cannot be assessed. Therefore, the phylogenetic relationships of genus #2 are rather difficult to ascertain, and we prefer to leave this genus in open nomenclature for the time being.

New genus and species #3

Occurrences: Khok Pha Suam, Ubon Ratchathani Province, Khok Kruat Formation.

Description

See Cuny et al. (2003) and fig. 2J-L.

Discussion

With only ten teeth at hand and no precise idea about the heterodonty pattern, the affinities of this new genus are rather difficult to decipher. Several characteristics recall the teeth of *Heteroptychodus:* the parallelogram to rectangular shape of the crown with a very high root showing a row of basal enlarged foramina, the basal surface of the root smaller than that of the crown, the presence of some ridges parallel to the longitudinal crest, and a crown which is arched in lingual and labial view. However, the small number of teeth currently available makes the hypothesis of a relationship with *Heteroptychodus* difficult to ascertain. On the other hand, similarities in root morphology (Compare fig. 2H and L) may indicate that genus #2 and #3 are closer to each other than to *Heteroptychodus*. Pending the discovery of more material, we therefore prefer to leave this genus in open nomenclature.

Discussion and conclusion

The Khorat plateau has yielded a rich hybodont shark fauna, although it was deposited in a nonmarine environment. Moreover, the diversity of the Sao Khua Formation is even higher than reported here as new material, not yet described, seems to indicate the presence of three new taxa belonging to the family Lonchidiidae. The shark diversity in the Sao Khua Formation is thus similar to that of the Khok Kruat Formation.

The Thai hybodonts display a wide range of possible diets according to their dentition. *Hybodus* spp. and genus #2 were probably opportunistic feeders while the grinding dentition of *Heteroptychodus*, genus #1, and genus #3 indicate more durophagous sharks. Finally, the cutting dentition of *Thaiodus* suggests a diet mainly consisting of large, soft-bodied preys. These sharks thus represented an important and diverse component of the freshwater ecosystems of the Khorat Plateau.

Genera #1, #2, and #3 are so far endemic to the Khorat Plateau and likely to have been infeoded to freshwaters, hence their endemism. *Hybodus* possesses a worldwide distribution, but the exact affinities of the Thai material appear unclear due to a lack of material, except for *Hybodus* sp. A, which clearly belong to a new species that appears again to be endemic to the Khorat Plateau. *Thaiodus* on the other hand is known both from the Khorat Plateau and Tibet, where it has been found in a deltaic environment (Cappetta et al., 1990). This shark was therefore able to tolerate some changes in salinity. Finally, *Heteroptychodus* has the largest distribution, having been recorded in Thailand, Japan, Kirghisia, and Mongolia (Yabe & Obata, 1930; Nessov, 1997; Tanimoto & Tanaka, 1998). The palaeobiogeographic distribution of the two latter taxa is difficult to explain if we consider these sharks as strictly confined to freshwaters. However, Maisey (1989) suggested that many hybodont genera (*Hamiltonichthys, Hybodus, Lissodus)* were in fact euryhaline. Such a mode of life would explain the distribution of *Heteroptychodus* and *Thaiodus* around the Asian continent, as it would explain how these hybodonts were able to follow the coastline to invade several freshwater systems. A similar strategy is seen today among the sawfish *Pristis perotteti*, which has colonized several lakes along the Atlantic coast of Central and South America (Thorson, 1982). Following a similar pattern, the spreading of *Thaiodus* and *Heteroptychodus-Asiadontus* would have been favoured by the Late Aptian sea transgression (Averianov & Skutschas, 2000). However, as these sharks are unknown outside Asia, they were probably unable to face open water.

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A REVIEW OF OSMOREGULATION IN FRESHWATER AND MARINE ELASMOBRANCHS

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Studies of osmoregulation in marine and particularly fresh water elasmobranchs have been intermittently reported in the literature over the last 80 years. Although there has been significant research on elasmobranch osmoregulation, no study exists uniting the previous work into a single comprehensive report. This study examines previous research in elasmobranch osmoregulation and presents the results in a single, comprehensive review, covering topics including body fluid (solute and solvent) volume and concentration variations, body fluid synthesis, retention and secretion, in different elasmobranch species, in different habitats, having varying nutritional states, and in different life history stages.

In elasmobranchs, blood and other body fluids are separated from the surrounding aqueous environment by permeable surfaces. Osmoregulation depends on the relationship between the solute to solvent concentrations of both the internal body fluids and the outside medium that surrounds the animal (Pang et al, 1977). Unless internal and external fluids have the same solute to solvent concentration, water will enter the body when its fluids contain a higher concentration of solute to solvent than does the water comprising the environment. In contrast, water will leave the body when the surrounding medium contains a higher concentration (Pang et al, 1977). Thus, marine animals face problems of dehydration and the elimination of excess salts while freshwater animals must conserve their salts and eliminate excess water (Pang et al, 1977).

Marine elasmobranchs have evolved the technique of reabsorbing and retaining urea and other body fluid solutes in their tissues so that serum osmolarity (solute/solvent concentration) remains just greater than that of the external seawater (Smith, 1931; Thorson, 1962; Poulsen, 1981). This greatly reduces their osmotic challenges so that they do not need to continuously drink seawater, as do teleosts. However, they still face the problem of a natural and continuous diffusion of salts into their bodies from the external seawater, where the concentration is higher. This is compensated for by salt excretion in the urine, by secretions of the rectal gland, and salt transfer at the gill epithelium (Haywood, 1973).

Fresh water elasmobranchs retain and synthesize less urea than that of their marine counterparts. Their body fluid solute concentrations are relatively low and urine is dilute and copious (Thorson et al, 1967; Thorson, 1970; Goldstein and Forster, 1971; Poulsen, 1981). This greatly reduces their osmotic problem of water retention. The freshwater stingrays of South America have abandoned retention of urea, they lack a functional rectal gland and they osmoregulate as much as do the freshwater teleosts (Thorson et al, 1967; Thorson, 1970; Goldstein and Forster, 1971; Thorson, 1976; Poulsen, 1981).

Review

Body Fluid comparison of freshwater and saltwater sharks

Marine elasmobranchs maintain serum osmolarity equal or slightly greater than that of their surrounding seawater environment and consequently suffer little or no osmotic loss of water (Pang et al, 1977; Poulsen, 1981). In dilute seawater or freshwater, elasmobranch serum osmolarity is reduced so that water does not continuously diffuse inward (Thorson et al, 1973).

Urea, TMAO, and other Ions:

In marine elasmobranchs, plasma osmolarity is high, in many cases higher than that of the surrounding seawater, largely because body fluid concentrations of organic nitrogenous compounds, such as urea and TMAO, are high. Bull sharks, *Carcharhinus leucas*, taken from marine waters have had mean serum urea levels of 356 mM/l and TMAO levels of 46.6 mM/l (Thorson, 1976; Thorson et al, 1973). Inorganic ions, both monovalent (sodium and chloride) and divalent (magnesium, sulfate and calcium), in marine elasmobranch body fluids are kept below seawater levels (Robertson 1975 and 1976). Holocephalans differ somewhat by having higher levels of sodium and chloride and lower levels of TMAO (Robertson, 1975 and 1976).

In freshwater elasmobranchs, plasma osmolarity is lower (but still higher than in teleosts) and body fluid concentrations of organic nitrogenous compounds such as urea and TMAO are relatively low (Smith, 1931; Thorson et al, 1973; Poulen, 1981). Thorson et al (1973) showed that *C. leucas* taken from fresh and estuarine waters had mean serum urea levels of 169 mM/l and TMAO levels of 13.2 mM/l respectively. Inorganic ions, both monovalent (sodium and chloride) and divalent (magnesium, sulfate, calcium), are kept at lower levels than those of marine elasmobranchs (Urist, 1962). Bull sharks that have moved into freshwater have urea levels about 40 % less than Bull sharks in the marine environment, magnesium levels about 37 % less and total sodium and chloride levels about 80 % less (Thorson et al, 1973).

Bony fish generally (except the coelacanth) have a plasma osmolarity only about one-third that of seawater (Pang et al, 1977).

Urea –biosynthesis and retention

Urea levels, which in high concentrations have shown to be largely responsible for the high osmolarity found in marine elasmobranchs, result from the difference between the rate of biosynthesis and excretion of the compound. The South American freshwater ray, *Potamotrygon*, has low rates of urea biosynthesis and has also lost the ability to reabsorb urea in the kidneys (Goldstein and Forster, 1971).

Hematocrit

The differences in hematocrit (percentage by volume of red blood cells) between freshwater and saltwater elasmobranchs are not significant. Furthermore, the freshwater environment does not elicit dilution of the plasma in species that can normally move between salt and freshwater (Thorson, 1961; Thorson et al, 1973)

Water content

Although there is a significant difference in the concentrations of body fluid solutes between salt and freshwater elasmobranchs, including in euryhaline species that can move between the two mediums, there is no shift or difference in the total water content and in the distribution of water among the fluid containing body compartments (Thorson, 1962)

Serum pH (Hydrogen ion concentration)

The serum pHs of freshwater, estuarine and marine sharks broadly overlap and are not significantly different from one another (Thorson et al, 1973).

Osmoregulatory functions of the Kidneys

The high level of urea in marine elasmobranch blood is maintained by the kidneys. Renal tubules are capable of reabsorbing urea, insuring that this important osmoregulatory compound is not wasted (Pang et al, 1977). Elasmobranchs that are adapted to dilute seawater increase the renal excretion of urea, thus effectively lowering their plasma urea levels and osmolarity; however as branchial loss of urea remains largely unaffected, the renal mechanism is likely more significant (Pang et al, 1977). The obligate freshwater rays, *Potamotrygon*, have abandoned renal reabsorption of urea (Goldstein and Forster, 1971)

Osmoregulation by the Rectal Gland

Marine elasmobranchs face the problem of a natural and continuous diffusion of salts into the body from the external sea water, where the concentrations are higher (Haywood, 1973). The rectal gland of marine elasmobranchs functions as a salt secreting mechanism (Conte, 1969; Oguri, 1964; Haywood, 1975); however, disturbances produced by the cessation of its activity can eventually be compensated for internally by other means that are as yet unknown (Burger and Hess, 1960; Conte, 1969; Haywood, 1975).

Freshwater elasmobranchs do not face the problem of continuous diffusion of salts into the body and in fact, the rectal gland of Bull sharks moving from salt to freshwater becomes regressive (Oguri, 1964). No functional rectal gland in the freshwater rays, *Potamotrygon,* has been found (Goldstein and Forster, 1971).

Osmoregulation of the Gills

Branchial elimination of salts in elasmobranchs has generally been considered of little importance. However, to keep serum salt levels low, two-thirds of total sodium and chloride excretion in elasmobranchs may take place through the gills (Payan and Maetz, 1973; Pang et al, 1977).

Low permeability of elasmobranch gills to urea (working in tandem with kidney reabsorption) maintains high plasma urea levels (Pang et al, 1977).

Osmoregulation in developing elasmobranchs, neonates and juveniles

Thorson and Gerst (1972) showed that serum urea levels of the uterine pups of the euryhaline Bull shark resemble those of the mother, and change as she passes from fresh to saltwater and back accordingly. Neonatal or juvenile Bull sharks can live in either fresh or marine water at birth and juvenile Bull sharks caught in totally freshwater habitats had serum parameters (including urea) similar to those of adult Bull sharks in the same environment (Thorson et al, 1973).

Diet

While the external medium controls the actual composition of body fluids for euryhaline elasmobranch osmoregulation, the level at which regulation between these fluids and the external medium occurs is also affected by the availability of metabolic urea, which is directly related to the availability of food (Haywood, 1973).

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REPRODUCTIVE ASPECTS OF FRESHWATER STINGRAYS (CHONDRICHTHYES: POTAMOTRYGONIDAE) IN THE BRAZILIAN AMAZON BASIN

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Introduction

The Potamotrygonidae Family is widely distributed in several river basins of the Neotropical region and encloses approximately 20 valid species (Rosa, 1985). Due to these species diversity and wide range of distribution, not all of them have been analyzed and data collecting / analysis is still under way. Freshwater stingrays have been considered completely adapted to freshwater environments because they have the ability to osmoregulate and reproduce in such habitats (Thorson *et al*, 1983).

Potamotrygonids present a reproductive mode described as matrotrophic viviparity with development of *trophonemata* (Thorson *et al*., 1983). The reproductive aspects of freshwater stingrays species have been poorly studied and limited knowledge related to the reproductive characteristics of this group is available.

The present study includes preliminary data of the reproductive aspects that have been observed for: *Plesiotrygon iwamae*, *Paratrygon aiereba*, *Potamotrygon motoro*, *P. orbignyi*, *P. schroederi*, *P. scobina* and *Potamotrygon* sp. C (*Potamotrygon* cf. *histrix* or "arraia cururu"). Further details are included in the complete publication (Charvet-Almeida *et al*., in press).

The observation of reproductive aspects of freshwater stingrays is essential to provide important data to contribute to the understanding of population dynamics of these stingrays and to provide adequate management and conservation plans.

Material and Methods

The species included in the present study were captured in two main fishing areas in the Brazilian Amazon Basin. Data related to the species *Potamotrygon motoro*, *P. schroederi*, *Potamotrygon* sp. C and *Paratrygon aiereba* was obtained from the State of Amazonas (Negro River Basin). Information about *P. scobina* and *Plesiotrygon iwamae* was collected in the State of Pará (Marajó Bay) and data concerning *P. orbignyi* was originated from both regions.

All specimens were captured with the assistance of local fishermen and observations were carried out using both fresh and fixed (10% formaldehyde solution buffered with borax) material. The terminology used and observations in the analyses were adapted from Dodd (1983), Thorson *et al*. (1983), Peres & Vooren (1991), Charvet-Almeida *et al*. (in press) and others.

The following macroscopic observations were performed on females organs: ovaries weight, length and aspect; number, diameter and weight of ovarian follicles; oviducts length and width; width, length, color and shape of the egg shell gland; uterine width, length and content; average size of uterine vili; number, stage of development, size and weight of embryos. Ovarian and uterine fecundity parameters were also observed. The following macroscopic details were recorded for male reproductive organs: testis size, weight and length; epididymides length and width; Leydig gland width; seminal vesicle width, length and number of sections; clasper length and level of calcification; % of clasper length in relation to disc width and presence or absence of seminal fluid. Slide preparations were used to check the presence of well-developed and active spermatozoids in the seminal fluid. Female and male specimens were considered juveniles, or adults, according to the criteria established in literature.

The analysis of the reproductive organs macroscopic characteristics provided information regarding reproductive aspects of the studied species in the study areas.

Results and Discussion

The species *Plesiotrygon iwamae*, *Paratrygon aiereba*, *Potamotrygon motoro*, *P. orbignyi*, *P. schroederi*, *Potamotrygon* sp. C seem to have well defined reproductive periods and their reproductive cycles closely related to the hydrologic cycle of the river basins that they inhabit. Estimates of pregnancy length and birth season were also obtained (Table 1).

Table 1 - Observations of gonadal maturation, copulation, pregnancy and birth periods (mo. = months; ? - indicates preliminary information that needs confirmation).

Species	Reproductive Aspects		Pregnancy	Birth	
	Gonadal maturation period (mo.)	Copulation in specific period? (season)	Gestation period (mo.)	Period (season)	Season length (mo.)
Plesiotrygon iwamae	2-3 (n = 51)	Yes, rainy (n = 51)	8 (?) (n = 15)	Transition dry to rainy (n = 15)	3 (n = 15)
Paratrygon aiereba	In study (n = 101)	In study (n = 101)	9 (?) (n = 2)	Rainy (n = 2)	4 (n = 2)
Potamotrygon motoro	3-4 (n = 110)	Yes, dry (n = 110)	6 (n = 18)	Rainy (n = 18)	4 (n = 18)
P. orbignyi	3-4 (n = 449)	Yes, rainy (n = 449)	6 (?) (n = 14)	Rainy (n = 14)	4 (n = 14)
P. schroederi	3-4 (n = 43)	Yes, rainy (n = 43)	6 (?) (n =5)	Rainy (n =5)	4 (n =5)
P. scobina	In study (n = 343)	In study (n = 343)	In study (n =31)	In study (n =31)	In study (n =31)
Potamotrygon sp. C	2 (n = 153)	Yes, rainy (n = 153)	3 (n =35)	Dry (n =35)	3 (n =35)

Preliminary information on ovarian and uterine fecundity and sexual maturity disc width are also included (Table 2).

Table 2 - Ovarian and uterine fecundity and sexual maturity disc width of the studied species (? - indicates preliminary information that needs confirmation).

Species	Fecundity		Sexual maturity
	Ovarian	Uterine (average)	Disc width males / females (mm)
Plesiotrygon iwamae	1-5 (n = 22)	1- 4 (2) (n = 15)	400 (n = 30) / 500 (n = 22)
Paratrygon aiereba	3 -5 (n = 45)	2 (2) (n = 2)	600 (n = 56) / 720 (n = 45)
Potamotrygon motoro	6 -11 (n = 50)	4 -11 (7) (n = 18)	390 (n = 60) / 440 (n = 50)
P. orbignyi	3 -7 (n = 162)	1-5 (2) (n = 14)	390 (n = 237) / 440 (n = 212)
P. schroederi	3 -7 (n = 20)	1-3 (2) (n = 5)	420 (n = 23) / 440 (n = 20)
P. scobina	1-13 (n = 107)	1-16 (6) (?) (n = 31)	350 (?) (n = 130) / 400 (?) (n = 107)
Potamotrygon sp. C	9 (n = 70)	1-5 (2) (n = 35)	160 (n = 83) /170 (n = 70)

The ovarian fecundity and number of embryos varied among potamotrygonid species and only the left ovary seemed to be functional. Abortions are very frequent and seem to occur when the pregnant females are exposed to stressful conditions. The embryos hardly ever survive in such circumstances despite their development stage. Males tend to present a smaller disc width size than females and this was observed in all species analyzed.

Conclusions

The present study was developed to help fulfill partially this lack of reproductive biology data from part of the Brazilian Amazon region. All species studied have defined reproductive periods with the following sequence of events: gonadal maturation, copulation, pregnancy, birth and resting. Each species analyzed presented a different reproductive periodicity. Reproductive aspects must be known and considered when elasmobranch conservation measures are required to assist decision-making in management procedures and help keep the populations of freshwater stingrays on safe ground.

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PRELIMINARY OBSERVATIONS ON THE FEEDING OF THE FRESHWATER STINGRAYS POTAMOTRYGON ORBIGNYI, POTAMOTRYGON SCOBINA AND PLESIOTRYGON IWAMAE (CHONDRICHTHYES: POTAMOTRYGONIDAE) IN THE COTIJUBA ISLAND REGION - PARÁ - BRAZIL

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Introduction

Freshwater stingrays are elasmobranchs restricted to the Neotropical Region included in only one family (Potamotrygonidae). This family is divided in three genera: *Potamotrygon* (18 species*), Plesiotrygon* (one specie) and *Paratrygo*n (one specie) (Mould, 1997). There are approximately 20 valid species of potamotrygonids (Rosa, 1985). The total number of species still is uncertain (Rosa et al., 1987) and many others can be discovered (Mould, 1997; Araújo, 1998; Charvet-Almeida, 2001).

Fish feeding habits aspects have received much importance in ichthyological research to help understand ecosystems functioning, in order to be able to manage them correctly (Zavalla-Camin, 1996). However, the number of papers about food habits of the Potamotrygonidae Family is reduced. Schomburgk (1843) is one of the first authors to mention the food intake strategies of the freshwater stingrays: hide in sandy substrate to surprise and capture prey. Achenbach & Achenbach (1976), in a paper about biological aspects of some species of freshwater stingrays (*Potamotrygon* spp.), carried out one of the first studies about food habits of Potamotrygonidae. Papers about feeding, based on the stomach content analysis, already became a routine in the study of fish ecology (Hyslop, 1980). Revisions of the methods to be applied have been developed and apparently there is consensus that more than one index must be used to obtain significant results with less bias (Hynes, 1950; Windell, 1968; Pinkas et al., 1971; Hyslop, 1980).

The Cotijuba Island is on the Marajó Bay, in the South of the Marajó Island, Pará State. This bay receives water discharge from the Tocantins River, some other tributaries (Guamá, Moju and Acará Rivers) and probably also receives part of the discharge of the Amazon River (Barthem, 1985; Barthem & Schwassmann, 1994). Seasonal differences of rainfall in the Amazon and the Tocantins basins cause a considerable variation in the volume of water carried by these rivers during one year, making the contact zone between the river and sea dislocate approximately 200 km in length (Egler & Schwassmann, 1962) and directly influencing the salinity in the Marajó Bay. The water salinity alterations of this bay become briny in the summer (June to December) and fresh in the winter (January to May) (Barthem, 1985). The Marajó Bay was chosen for the present study on feeding, because of the presence of freshwater stingrays in this region. This research contributed to the knowledge of the feeding biology of three species of freshwater stingrays (*Potamotrygon orbignyi*, *Potamotrygon scobina* and *Plesiotrygon iwamae)* in the Marajó Bay Region. Data obtained included qualitative and quantitative evaluation of food items found in the stomachs, verification of the degree of digestion of all food items and observation of the repletion level of the stomachs.

Material and Methods

Local fishermen and research team captured the specimens in May, August and October of 2000 by local fishermen and research team. The stingrays were captured with longlines (200 to 300 hooks in each line) and beach trawls (100 m long, mesh size of 70 mm). Each specimen captured received a number tag before being weighed and dissected. Both extremities of the stomachs were tied before being removed. Stomachs were fixed in formaldehyde 10% solution for few days and then were washed with current water before conserved in alcohol (70%). Each food item was identified in up to Order and Family category whenever possible. The

following indexes were calculated for each food item: Frequency of Occurrence; Numerical Percentage; Weight Percentage. From the attainment of this information the Relative Importance Index (IRI) was calculated for each food item (Pinkas et al., 1971). This index relates the frequency that each item appears in the diet with the proportion of the same item in the stomach content. The calculation of the IRI is based on the following calculation: IRI **= % FO x (% W + % N). The Index of Relative Importance (IRI) was transformed into percentage (%IRI) for better interpretation of the data according to Cortés (1997). The Repletion Level of the stomachs was observed and the following values were attributed: 0 = empty; 1 = ¼ filled; 2 = half full; 3 = ¾ filled; 4 = full.** Analysis of the foods items Degree of Digestion was also observed and the following values were attributed: 1 = not digested; 2 = only external parts partially digested; 3 = external parts and muscular mass partially digested; 4 = only the axial skeleton and part of the muscular mass remained; 5 = only fragments remained (Zavalla-Camin, 1996).

Results

The stomachs of 40 specimens of *Potamotrygon orbignyi*, eight of *Potamotrygon scobina* and four of *Plesiotrygon iwamae* were analyzed. The results observed for Degree of Digestion , Repletion Level and items found in the analyzed stomachs are shown in Tables 1, 2, 3, 4, 5 and Figure 1.

Table 1 - Degree of Digestion of food items found in *Potamotrygon orbignyi*, *Potamotrygon scobina* and *Plesiotrygon iwamae* in Cotijuba Island.

SPECIES	DIGESTION LEVEL	ITEMS	%
Potamotrygon orbignyi	1	4	0,21
	2	1355	72,85
	3	505	26,67
	4	5	0,27
	5	0	0
	Total	1869	100
Potamotrygon scobina	1	4	0,81
	2	432	87,63
	3	57	11,56
	4	0	0
	5	0	0
	Total	493	100
Plesiotrygon iwamae	1	15	15
	2	85	85
	3	0	0
	4	0	0
	5	0	0
	Total	100	100

Table 2 - Repletion Level of the stomachs analyzed of *Potamotrygon orbignyi*, *Potamotrygon scobina* and *Plesiotrygon iwamae* captured in the Cotijuba Island.

SPÉCIES	REPLETION LEVEL	STOMACHS NUMBERS	%
Potamotrygon orbignyi	0 (empty)	4	10
	1 (1/4 full)	13	32,5
	2 (1/2 full)	10	25
	3 (3/4 full)	7	17,5
	4 (full)	6	15
	Total	40	100
Potamotrygon scobina	0 (empty)	0	0
	1 (1/4 full)	3	37,5
	2 (1/2 full)	4	50
	3 (3/4 full)	1	12,5
	4 (full)	0	0
	Total	8	100
Plesiotrygon iwamae	0 (empty)	0	0
	1 (1/4 full)	2	50
	2 (1/2 full)	2	50
	3 (3/4 full)	0	0
	4 (full)	0	0
	Total	4	100

Table 3 - Food items found in the stomachs of *Potamotrygon orbignyi.*

Common name	Order	Family	% FO	% N	% W	IRI	% IRI
Insect larvae 1	Diptera	Chironomidae	42.5	44.3	23.5	2883	32.3
Insect larvae 2	Diptera	?	7.5	0.9	0.4	10	0.1
Shrimp	Decapoda	Palaemonidae	10.0	1.0	3.5	45	0.5
Water lice	Isopoda	Sphaeromatidae	47.5	53.7	72.3	5989	67.1
Water lice 2	Isopoda	?	2.5	0.1	0.3	1	0.01
Total	-	-	100	100	100	8927.	100

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Table 4 - Food items found in the stomachs of *Plesiotrygon iwamae.*

Common name	Order	Family	% FO	% N	% W	IRI	% IRI
Shrimp	Isopoda	Palaemonidae	75	78	98	13205	88
Vegetable (litter)		?	75	22	2	1795	12
Total		-	150	100	100	15000	100

Table 5 - Food items found in the stomachs of *Potamotrygon scobina*.

Common name	Order	Family	% FO	% N	% W	IRI	% IRI
Water lice	Isopoda	Sphaeromatidae	62.5	96.7	29.6	7898	80.8
Shrimp	Decapoda	Palaemonidae	25	2.5	68.1	1763	18.0
Vegetable (litter)		?	37.5	0.8	2.3	115	1.2
Total		-	125	100	100	9777	100

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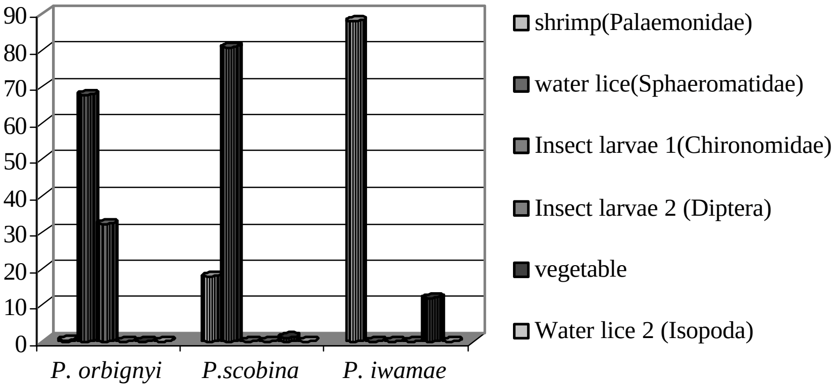

- Figure 1 - Percentage of IRI (%IRI) of food items found in the stomachs of *Potamotrygon orbignyi*, *Potamotrygon scobina* and *Plesiotrygon iwamae* in Cotijuba Island.
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Discussion

The present study indicated that most of the analyzed stomachs contained little digested food items. The species *P. orbignyi*, *P. scobina* and *Plesiotrygon iwamae* presented 73%, 88% and 85%, respectively, of food items digested to level 2 (only external parts partially digested). These results indicated that the majority of these freshwater stingrays had apparently fed a short time before being captured. The Repletion Level analysis pointed that few stingrays were observed without food items in their stomachs. In terms of stomach Repletion Level, about 1/3 of *P. orbignyi* specimens were placed under level 1 (1/4 filled), and the other 2/3 were in level 3 or 4 (almost full or full). Half of the *P. scobina* specimens observed had stomachs in level 2 (1/2 full). The Repletion Level of the remaining specimens was considered as in level 1 (37,5%) and 3 (12,5%). Only four *Plesiotrygon iwamae* were examined and half of them had stomachs in level 1 and the other half in level 2. Considering the capture occurred in different times of the day, this data suggests that these stingrays feed very often and mainly when they are in shallow waters near the beach. Charvet-Almeida (2001) indicated that most of the *Plesiotrygon iwamae* stomachs (73,5%) analyzed were included in Repletion Level 1, suggested that this species probably is constantly searching for food, does not have a specific feeding time associated to photoperiods and that food intake occurred associated with tidal cycles. The present study presented results similar to these and eventually the same hypothesis could be valid for the specimens observed in the Cotijuba Island region.

Most *Potamotrygon* specimens captured in the beaches of the study area were juveniles, while *Plesiotrygon iwamae* specimens were all adults. It is possible that juveniles of *Potamotrygon* spp. preferred beach areas due to the low intensity of the water current, protection and food availability. The results of the present study indicated that Arthropods are the most important food item for the freshwater stingrays in the Cotijuba Island region. The food item analysis indicated that *P. orbignyi* preferred crustaceans, especially Sphaeromatidae water lice (%IRI = 67%) and Chironomidae insect larvae (%IRI = 32%). The stomach content analysis of *Potamotrygon scobina* pointed a feeding preference for crustaceans, especially for Sphaeromatidae water lice (%IRI = 81%) and Palaemonidae shrimps (%IRI = 18%). The results for *Plesiotrygon iwamae* suggested a preference for Palaemonidae shrimps (%IRI = 88%). The feeding habits and food intake of the studied stingray species in the Cotijuba Island suggests that they may be classified as heterogeneous, stenophagic and carnivorous. Shrimps were the dominant food category for *Plesiotrygon* *iwamae*. Water lice dominant for *Potamotrygon scobina* and *Potamotrygon orbignyi*. The dominant presence of shrimps and water lice might be related to the abundance of these crustaceans in the Amazon estuary region. The capture of shrimps in the Amazon estuary was observed as part of the regional fishery practiced in this area (Aragão et al., 2001).

The presence of crustaceans in the diet of other species of freshwater stingrays was mentioned by others authors (Achenbach & Achenbach, 1976; Lasso et al., 1996). Lasso et al. (1996) observed that *Paratrygon aiereba* has a food preference for fish and crustaceans and that other freshwater stingrays (*Potamotrygon* spp.) feed on shrimps (*Macrobrachium* spp.) and aquatic snails of the genus *Pomacea*. The description of *Plesiotrygon iwamae* (Rosa et al., 1987) pointed this species as being predominantly piscivorous; however, Charvet-Almeida (2001) indicated this species as feeding mainly on crustaceans (predominantly shrimps) and fish in the Marajó Bay Region. Lasso et al., (1996) studied the diet of *Potamotrygon orbignyi* in the Venezuelan *llanos* region and considering the Frequency of Occurrence index indicated that *Potamotrygon orbignyi* had an insectivorous preference, with the predominance of Chironominae (Subfamily Tanypodinae Order Coleoptera). Many Chironomidae insect larvae (% IRI = 32%) were also found in the stomach contents of *Potamotrygon orbignyi* captured in the Cotijuba Island. The occurrence of plant fragments (litter) in the stomachs of *Potamotrygon scobina* and *Plesiotrygon iwamae* probably is related to the accidental ingestion of these items by these freshwater stingrays when they use suction to capture their preys.

Conclusion

The Repletion Level was relatively high for the three studied species. The Degree of Digestion of food items analyzed was relatively low indicating they probably they had been recently ingested. The freshwater stingray *Potamotrygon orbignyi* seemed to prefer crustaceans and insects. On the other hand, *Potamotrygon scobina* and *Plesiotrygon iwamae* apparently demonstrated a feeding preference for crustaceans, especially shrimps and isopods. The results suggest that the stingray species observed apparently present a heterogeneous, stenophagic and carnivorous diet in the Cotijuba Island region.

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CONSERVATION STATUS OF FRESHWATER STINGRAYS
(CHONDRICHTHYES: POTAMOTRYGONIDAE)
IN THE BRAZILIAN AMAZON

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Abstract

Potamotrygonids are Elasmobranchs that share some features with marine forms: low fecundity, late maturation and slow growth. Besides this, constrains of freshwater environment become potamotrygonids species more vulnerable to fisheries than the marine counterparts. Potamotrygonidae are fisheries to ornamental purpose for more than two decades at Brazilian Amazon. Artisanal fisheries for food purposed also capture potamotrygonids. Commercial fisheries with trawl net use to catch freshwater stingrays as bycatch. The main problems involving fishery and conservation in this group are species incorrect identification, and lost biological information.

Introduction

South American freshwater stingrays are included in a single Family (Potamotrygonidae) and represent an important part of the Neotropical ichthyofauna. Potamotrygonids belong to the only group of Elasmobranchs completely restricted to freshwater habitats (Brooks et al, 1981; Compagno &

Cook, 1995) and are widely distributed in most river systems of tropical and subtropical South America draining to the Atlantic Ocean (Rosa, 1985).

The life history of potamotrygonids exhibits some features similar to the ones found in marine Elasmobranchs, such as: low fecundity, late maturation and slow growth (Camhi et al., 1998). Besides this, constrain to freshwater environments (including habitat reduction during the dry season) makes these stingray species more vulnerable to fishing activities than their marine counterparts. Some freshwater stingrays are endemic and have a restricted range with a relatively low limit of tolerance to both natural and anthropogenic impacts, such as ecotourism, habitat deterioration and loss (Compagno & Cook, 1995; Barcellos, 1996; Araújo, 1998; Charvet-Almeida, 2001, Araújo et al., 2004).

Potamotrygonids have been fished for different purposes for several decades in the Brazilian Amazon region. Only recently, baseline and historic data on species biology and abundance began to be collected. Part of this information is being used as guidelines in the management plan of this fishing activity in Brazil (Araújo et al, 2004). Freshwater stingrays present high polychromatism (Almeida, 2003) that often leads to species misidentification and may cause loss of accurate biological information (Charvet-Almeida et al., 2002). In order to be sustainable, elasmobranch fisheries must take into account the biological capacity of the species, which requires basic taxonomic knowledge (Camhi et al, 1998). In this sense, lots of effort must be put to help solve some of the potamotrygonid species identification problems.

Many species of freshwater stingrays occur in more than one country (Araújo et al, 2004) and probably there are stock differences that require distinct management plans. The lack of adequate management in some regions associated to habitat damage and deterioration may be factors that lead to population declines as has been suggested by Camhi et al. (1998) for sharks species. Considering these aspects, isolated species protection measures and fishery management will not offer sufficient / good support to develop conservation programs for freshwater stingrays, except for a few endemic species. This report provides a brief summary of some fishery aspects and conservation status of potamotrygonid species in the Brazilian Amazon.

Material and Methods

The present study included data from two different areas in the Brazilian Amazon region. Area A is included in the Barcelos Municipality (0°30’ S, 63°12’ W), located in the Rio Negro Basin and corresponds to the main fishing area of the ornamental fishery industry in the Amazonas State. Area B included two islands (Cotijuba 01°15’30’’ S, 48°33’30’’ W; and Colares 00°55’27’’ S, 048°17’21’’ W) located in the Marajó Bay, Amazon estuary, State of Pará, and is known for its high abundance of stingrays species (Figure 1).

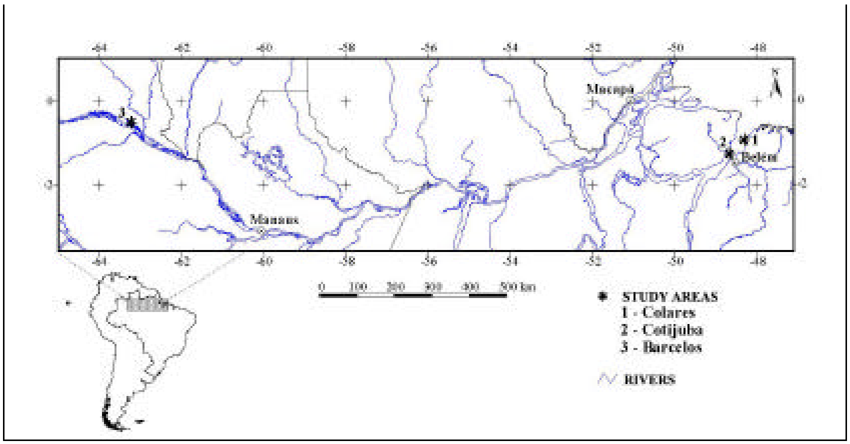

Figure 1: Map of the two main study areas where points 1 and 2 correspond to Area B and point 3 to Area A.

In both sites, fishing trips were conducted in collaboration with local fishermen, during the fishing season (Area A) and all year round (Area B). Several specimens of freshwater stingrays were collected, dissected and had biological parameters observed. Environmental variables, such as temperature (°C), dissolved oxygen (mg/l), conductivity (µS), and pH data were also collected at each sample site. During the research trips several fishermen were interviewed to provide complementary information. Capture effort data (CPUE) and post-capture mortality were calculated only for Area A, considering the ornamental fishery (Araújo, 1998). The main fishery characteristics of each area, sampling method to obtain the specimens and basic environmental differences are indicated in Table 1.

Table 1: Fishery, sampling and main environmental characteristics of each studied area.

Fishery Characteristics	Area A	Area B
Capture methods.	Hook and line, drill net with flashlights and battery, harpoon, night captures only.	Longlines, hook and line, beach seine, bottom trawl net, day and night captures.
Purpose of the captures.	Ornamental and "negative fishery".	Food source, medicinal and occasionally ornamental.
Months of capture (fishing season).	August - April.	All year round.
Water quality.	Poor black water, acid, warm and low in dissolved oxygen.	White water, warm, slightly acid (pH 5-6), oxygen rich, with seasonal salinity influence.
Bottom substrate.	Sandy and muddy.	Sandy, muddy and rocky.
Duration of each research trip (main season of sampling).	10 days (August - April)	5 days (all year round).

Results and Discussion

Freshwater stingrays presently are not among the main target species for the ornamental fish industry, nor for the commercial fishery fleet in the Brazilian Amazon region. The species observed in the present study were: *Plesiotrygon iwamae*, *Paratrygon aiereba*, *Potamotrygon motoro*, *Potamotrygon orbignyi*, *Potamotrygon schroederi*, *Potamotrygon scobina* and *Potamotrygon* cf. *histrix* (*Potamotrygon* sp. C or "cururu" freshwater stingray). The different species studied seemed to have distinct specific uses according to each area (Table 2).

Table 2 - List of species of Potamotrygonidae inevaluation by the current study and their uses as natural resource.

Species	Type of use	Area
Plesiotrygon iwamae	Food, medicinal and ornamental purposes.	B
Paratrygon aiereba	Food, ornamental and medicinal purposes.	A and B
Potamotrygon motoro	Ornamental, food and medicinal purposes	A and B
P. orbignyi	Ornamental, food and medicinal purposes.	A and B
P. schroederi	Ornamental purpose.	Just in A
P. scobina	Food, ornamental and medicinal purposes.	Just in B
Potamotrygon. cf. *hystrix*	Ornamental purpose.	Endemic to A

Potamotrygonids have been captured for ornamental purposes for over two decades in the Brazilian Amazon (Araújo, 1998). The exportation of theses species represents less than 1 % of all fishes exported from Amazonas and Pará States. Nevertheless, these stingrays have a significant role in the ornamental trade since often the exportation of other species takes place only if a few freshwater stingrays are included in the deal. Potamotrygonids also can raise the exports of other traditional species, such as *Paracheirodon axelrodi* (Araújo et al. 2004). Nowadays freshwater stingray exports are controlled by a Brazilian Environmental Agency federal regulation (IBAMA, number 036/2003), which determines a list of six species of potamotrygonids that are allowed to be exported according to a yearly species-specific quota system. Only recently catch and effort dates of Potamotrygonidae fisheries for the ornamental trade began to be collected.

In Amazonas State, where 10.000 units of stingrays are exported annually, the fishery effort is concentrated on *Potamotrygon* cf. *hystrix* stocks (Figure 2).

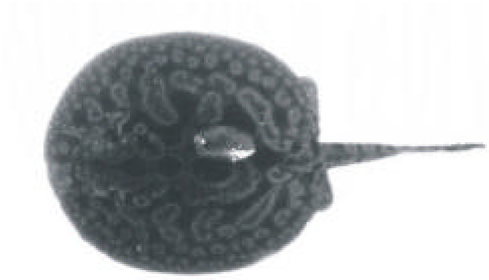

Figure 2 - *Potamotrygon* cf. *hystrix*, commonly known as “arraia cururu” and presently being described (Photo: Maria Lúcia G. Araújo).

This species is one the smallest species of freshwater stingrays, with maximum disc width of 500 mm. It is endemic of the Rio Negro Basin and high densities of individuals are observed in island and flooded forest. Approximately 60% of the captures of this species are concentrated in only one tributary of the Rio Negro. The CPUE data for this species in this area does not exhibit a direct relationship with fishery effort, neither exportation demand. Observations indicated that the effort pressure on the stocks varies according to the level of the river (Table 3).

Table 3 - CPUE data of freshwater stingray captures in area A.

Year	Months of low water period	CPUE
1998-1999	2	10,26
1999-2000	4	34,62
2000-2001	3	26,15

This species vulnerability is associated to habitat accessibility by traditional ornamental fishermen and to opportunistic commercial fishermen fishing for food. The average post-capture mortality of this species ranges between 2 - 5 %. *Potamotrygon* cf. *hystrix* represents 31,25 % of all freshwater stingrays legally exported from Brazil Its captures are monitored and the number of individuals exported annually is controlled to avoid stocks depletion.

Potamotrygon motoro (Figure 3) is one of the most fecund stingrays and presents a wide distribution in all Neotropical region. Its captures and exportations are monitored too. There is no evidence of reduction in the abundance in this species, but a strict export control must exist to avoid misidentification of this species with the endemic *P. leopoldi* and *P. henlei*. The post-capture mortality is estimated between 10 - 12 %.

Figure 3: *Potamotrygon motoro* (Photo: Mauricio P. Almeida).

Potamotrygon orbignyi (Figure 4) is a well-distributed species in all Amazon Basin. It is a highly polychromatic stingray and sometimes difficult to be recognized, especially due to the existence of some faded color patterns. The evaluation of CPUE for this species did not show reduction in abundance and post-capture mortality ranges from 8 - 10 %.

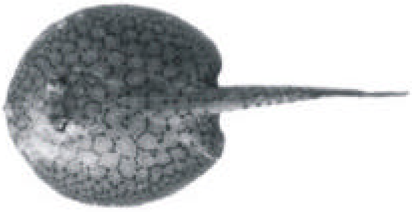

Figure 4 - *Potamotrygon orbignyi* (Photo: Patricia Charvet-Almeida).

Potamotrygon schroederi (Figure 5) is a rare stingray with not many exports records. This is due partially because this species has the highest post-capture mortality (10 - 12 %) of all legally exported stingrays. The capture and exportation of this species must also be controlled because it might be irregularly exported as *Potamotrygon* cf. *hystrix.*

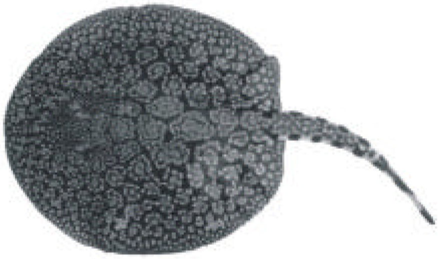

Figure 5 - *Potamotrygon schroederi* (Photo: Maria Lúcia G. Araújo).

Artisanal fisheries also capture potamotrygonids as a food source. These captures are restricted to some areas, such as the Amazon estuary (Area B), where longlines are used as the main fishing gear. This stingray fishery is considered of low economic value. Potamotrygonids are not the main target species but are never discarded when caught and are sold at local fish markets. At least three species are caught in this fishery: *Plesiotrygon iwamae*, *Potamotrygon orbignyi* and *P. scobina* (Charvet-Almeida, 2001; Almeida, 2003).

Incidental capture by commercial fisheries with trawl net happen along Solimões-Amazonas River, and it has introduced an unknown mortality rate on freshwater stingrays stocks.

The negative image of freshwater stingrays cause the “negative fishery” The effect of disturbance from ecotourism operations has removed at least 21 000 individuals Potamotrygonidae (mainly *Potamotrygon orbignyi* and *Paratrygon aiereba*) at Rio Negro Basin This kind of fishery is developed by some ecotourism companies to avoid accidents with freshwater stingrays. It is an unregulated activity because is not considered “fishery” by Brazilian Environmental Agency (IBAMA) (Araújo et al 2004).

Brazil is in South America the only country that has a specific regulation to control the export of freshwater stingrays species to ornamental trade. Two of the three endemic species permitted to be exported *Potamotrygon henlei* and *P.*

leopoldi are in basins where dam projects are planning to be developed in the next years. The other endemic species *Potamotrygon* cf *hystrix* will need soon National protection of its habitat because in this range ecotourism projects and dragging activities of Rio Negro will compromise its abundance Habitat damage or loss, can deplete freshwater stingrays population more severity than fisheries. (Araújo et 2004)

A critic question at ornamental fish trade is mortality post-capture. The values estimated for freshwater stingray depends of the species rusticity, fishermen practice and exportation area facilities.

The causes of mortality can be categorized as high density in the tanks or plastic boxes, oxygen and pH requirements, and skin lesions (inflammatory oedema) around the mouth, the anterior margin of the disc and pelvic fins. *Potamotrygon* cf *hystrix* exhibit the smallest mortality rate what justify its high capture rates, the opposite happens with *P. schroederi*

Many species of freshwater stingrays occur in more than one country, this is the case of *P. motoro*, *P. orbignyi*, *P schroederi*, *Paratrygon aiereba* and *Plesiotrygon iwamae* but until now there are no shared stocks. Despite the fact that Brazilian scientist has proposed a National Plan to management the capture of Potamotrygonidae from commercial vessels, and practice of negative fishery, any effective measured has been considering by IBAMA

Conclusions

The importance to ornamental fish industry is having stingray to offer to ornamental trade, cannot be ignored because this activity employs at least 10.000 families in Brazilian Amazon. The lack of adequate identification of some freshwater stingrays species is a problem that must be solve soon, because the population models to Elasmobranch species require a species-specific data on life cycle parameters. A conservation program to freshwater stingrays species to be well succeed must consider not only the regulation of fish activities but the habitat deterioration and loss, as well the development of ecotourism.

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