

# The Syngnathid Biology International Symposium



**Sven Lovén Centre for Marine Research  
Kristineberg, Sweden**



**Sponsored by the Nordic Marine Academy**



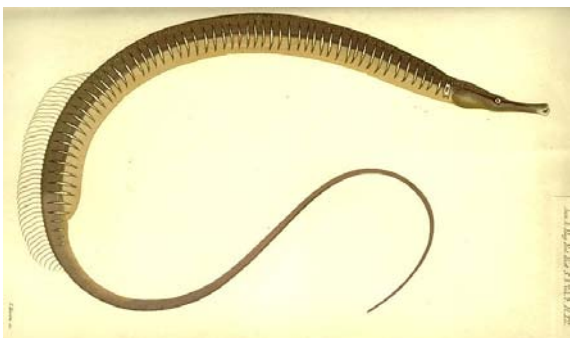
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## Välkommen!

It is our great pleasure to welcome you to the first Syngnathid Biology International Symposium held in Kristineberg, Sweden. We look forward to hosting you and hope that it will be a fantastic experience for all.

Sincerely,  
The conference organizers  
Kenyon Mobley, Gunilla Rosenqvist & Anders Berglund



*Our logo has been fashioned from a public domain print of Entelurus aequoreus L., the snake pipefish. This species is commonly found in NE Atlantic waters and the west coast of Sweden.*

*Source: J. Leckenby, Esq. 1858. On a species of pipefish (Syngnathus aequoreus?) lately found at Scarborough. Annals and Magazine of Natural History. 416*



## Schedule at a glance

	Mon April 25	Tuesday April 26	Wednesday April 27	Thursday April 28	Friday April 29
0730-0830		<i>Breakfast</i>	<i>Breakfast</i>	<i>Breakfast</i>	<i>Breakfast</i>
0900-1030		<b>Morning Session:</b> Sexual selection and Mating systems	<b>Morning Session:</b> Evolution, Sexual Selection & Hormones	<b>Morning Session:</b> Physiology & Hormones	<b>Morning Session:</b> Conservation & Climate Change
1030-1100		<i>Coffee/fika</i>	<i>Coffee/fika</i>	<i>Coffee/fika</i>	<i>Coffee/fika</i>
1100-1220		<b>Morning Session cont.:</b> Sexual selection and Mating systems	<b>Morning Session cont.:</b> Evolution, Sexual Selection & Hormones	<b>Morning Session cont.:</b> Physiology & Hormones	<b>Morning Session cont.:</b> Conservation & Climate Change
1220-1330		<i>Lunch</i>	<i>Lunch</i>	<i>Lunch</i>	<i>Lunch</i>
1330-1450		<b>Afternoon Session:</b> Sexual selection and Mating systems	<i>Free time/Excursions</i>	<b>Afternoon Session:</b> Conservation	<b>**End**</b>
1450-1520		<i>Coffee/fika</i>		<i>Coffee/fika</i>	
1520-1630		<b>Afternoon Session cont.:</b> Sexual selection and Mating systems		<b>Afternoon Session cont.:</b> Conservation	
1630-1800		<b>Poster session (main entrance)</b>			
1800-2000	<b>Reception &amp; Pizza Dinner (main entrance)</b>	<i>Dinner</i>	<i>Dinner</i>	<b>Banquet Dinner starts at 1830</b>	
2000-2200			<i>Workshop: IUCN Red Listing for syngnathids</i>		

All meals are served in the Kristineberg Massen except the pizza dinner/reception that will be held in the main entrance to Kristineberg in the main building.

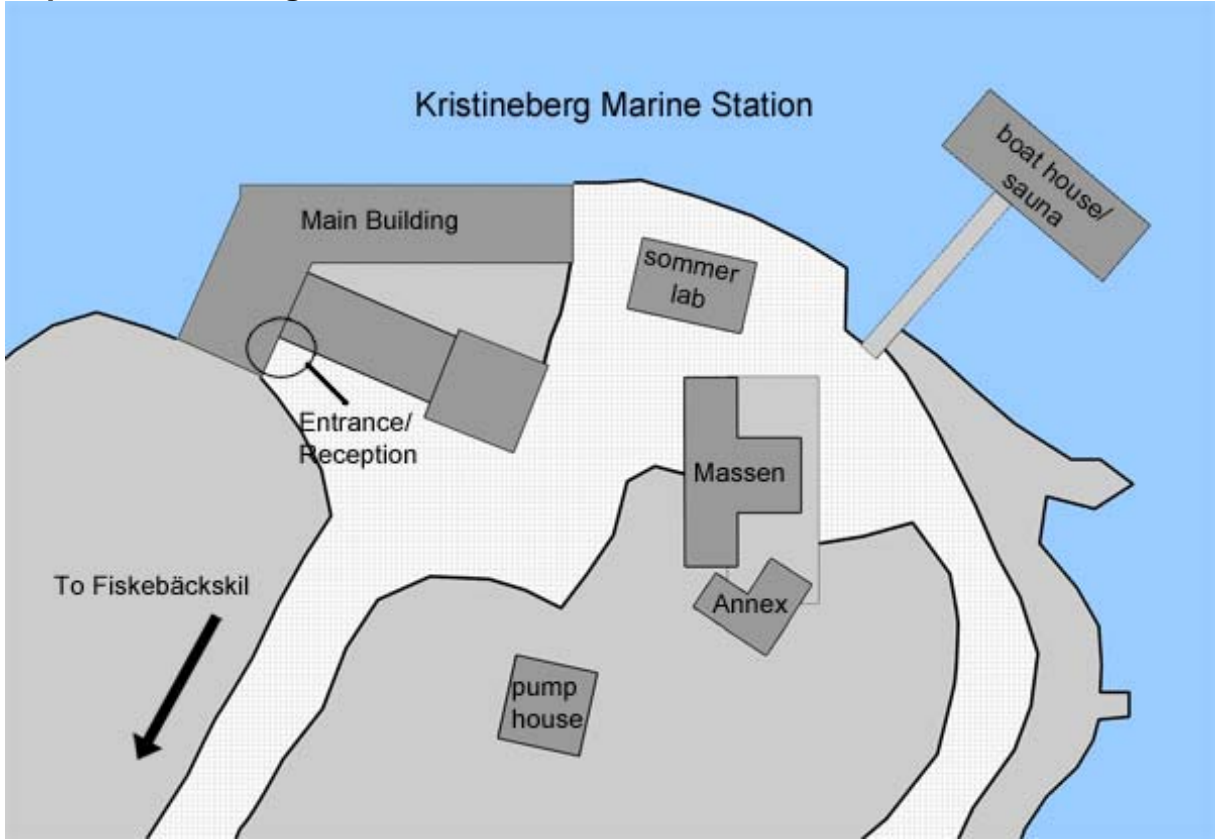
All talks and workshop will take place in the main lecture hall in the main entrance of Kristineberg

## List of Participants

<b>Name</b>	<b>Organization</b>	<b>email</b>
Ahnesjö, Ingrid	Uppsala University	ingrid.ahnesjo@ebc.uu.se
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**Maps of Kristineberg and Fiskebäckskil**

**Map of Kristineberg**



## Map of Fiskebäckskil





## **Important Information**

### **ACCESS TO BUILDING/CODE**

Access to the main building and Mässen will be restricted during the hours of 1630-0730. If you are staying at the station, you will be issued a code that will allow access during these times.

### **ALARM**

There is an alarm in the main building. The alarm is armed automatically from midnight (2400) until 0600, or until the first person switches it off in the morning. If you arrive in the evening or over the weekend, please contact reception or your supervisor next day for further instructions on how to use the alarm.

### **CHECK IN/CHECK OUT**

Check-in at 1400 / check-out by 1000 on the day of departure.

### **EMERGENCY**

Fire-extinguishing equipment, emergency exits and assembly points are marked. First-aid supplies are available in each building. If you require assistance from the rescue services or police, call the **emergency number (0) 112**.

### **INTERNET ACCESS**

There are several computers available for use on the second floor of the main building in Kristineberg slightly to the right at the top of the spiral staircase (immediately above the reception desk). Please be courteous and limit your usage to 15 minutes if others are waiting to use the machines.

#### *WIFI Users*

You can connect to Internet via two different wireless networks at Kristineberg Göteborgs Universitet and eduroam. eduroam (EDUcation ROAMing) gives you a secure connection (radiotraffic is encrypted) and access to a lot more services. Users within the eduroam federation can connect using the same credentials (for instance, username and password) the users would use if they were at their home university. The home university (ID-provider) provides support for eduroam. You can get more information about eduroam at <http://it.gu.se/it/guwan> and <http://www.eduroam.se>.

Göteborgs Universitet uses web logon, has no encryption of the radio traffic and is easy to use as a guest at the university. You will get access to a few key services such as seeking information on the Internet and a couple of encrypted protocols. If you are a guest and need a guest account, contact Marita Nyberg ([marita.nyberg@loven.gu.se](mailto:marita.nyberg@loven.gu.se)) at the reception.

Guests at the University are obliged to follow the regulations for the use of University of Gothenburg's IT facilities. You can find the regulations and more information at <http://info.slc-k.gu.se/it>.

### **KITCHEN INFORMATION**

You can store and cook food in the kitchenette in the massen. Please mark your food and dispose of all leftover food at the end of your stay. You are responsible for washing up and clearing the kitchen immediately after cooking, and everyone has a

joint responsibility for ensuring that the oven, surfaces and tables are wiped and kept clean.

### **LAUNDRY**

There is a laundry room on the ground floor of the massen. One wash costs 10 SEK and requires a token that you can get at reception. There is a drying room adjacent to the washing room to hang laundry.

### **RECEPTION**

Hours of operation is between 0800 and 1600, with a lunch break between 1230-1300. Reception will provide you with keys/codes to your accommodation. They sell lunch vouchers, stamps and postcards, among other things. They take payment for your stay and can answer any questions you might have.

### **SAUNA**

There is a sauna in the boathouse which requires a key that is available at the reception. There are instructions in the boathouse for its use. Clean up after yourselves and no glass bottles are allowed in the sauna. Also, be aware that like most saunas in Scandinavia, clothing is optional.

### **SMOKING**

Smoking is strictly prohibited in any of the buildings and around the boat dock. Please extinguish cigarettes in appropriate bins.

## Excursions

### Ferry to Lysekil

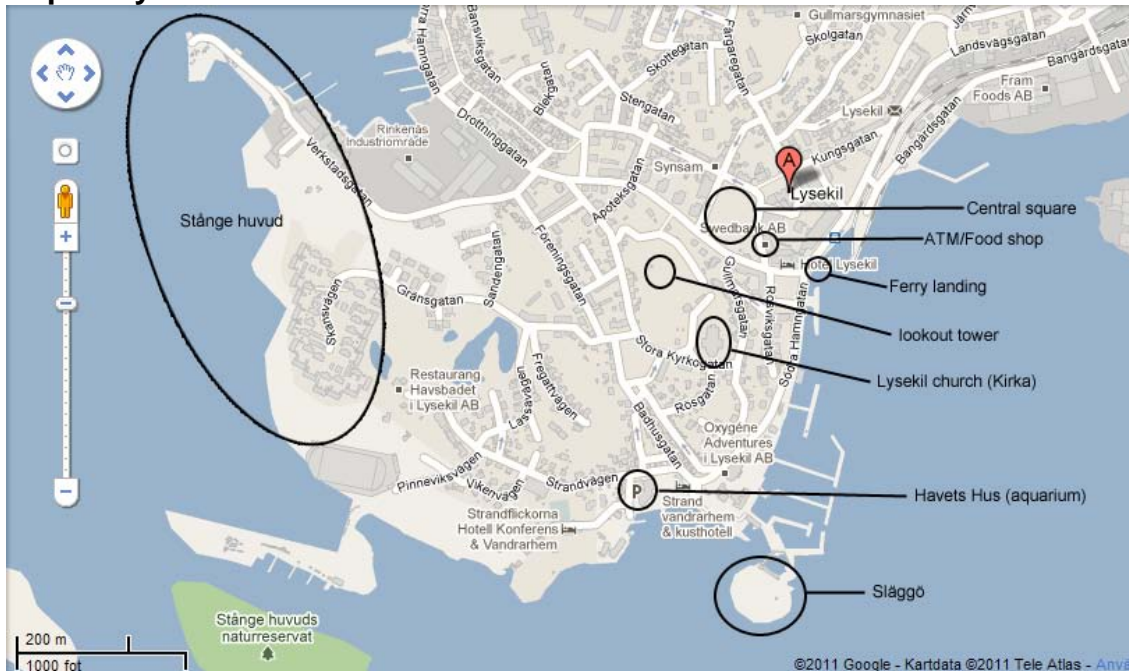
**Warf/Town Center** Along the warf and in the town center you will find a number of shops, pubs and eateries. Good places to buy souvenirs, gifts, books or just relax with a cup of coffee.

**Old Church/Lookout Tower** If the weather is nice, we recommend a leisurely walk up to the old church and lookout that allows great views of Lysekil. The lookout is behind the church.

**Släggö/ Stånge huvud** For the more adventurous types, these two locations offer great hiking and pick nick locations on the granite rock formations. Great views of the sea and fantastic sunsets (weather permitting of course).

**Havets Hus** - This is a public aquarium that is worth a look. To find Havets Hus turn left as you leave the ferry and follow signs marked along the road.

### Map of Lysekil



### Fiskebäckskil/Östersidan

The traditional houses of Fiskebäckskil and Östersidan give a sense of timelessness in these turn-of-the-century fishing villages. Take a short hike up to the windmill, navigate the cobblestone paths and find the oldest grave in the graveyard of the fisherman's church. The two main places to eat, the Brygghuset and the Gullmarstrand hotel are not open this afternoon. You can take a short walk over to sleepy Östersidan and visit Uppsala's marine station Klubban. On the road between the two towns are a few pizza cafes and Skäftöhallen, the local supermarket.

### Stockevik/Grundsund

Grundsund is a quaint old fishing village similar to Fiskebäckskil but on the southern end of Skäftö. You can take bus 845 in the direction toward Grundsund (closest bus stop is by the old church in Fiskebäckskil). There are some small shops and cafes but nothing fancy. There also is a well marked hiking trail originating from the upper fields on Kristinebergsvägen that lead all the way to Grundsund. It's a gentle loose-

gravel path that winds its way through the small village Stockevik, past a cholera graveyard and on to Grundsund. The length of the path to Grundsund is 5km in one direction so you may want to do only part of it before turning around. If you brought some running shoes and feel strong, go for it!

### **Hiking on Skaftö**

There are extensive hiking trails that cover the island of Skaftö. Some trails are well marked and some are not. The notion of “private property” does not apply to most of Sweden and you are free to wander anywhere on the island but stay on paths and roads when in residential areas. There are maps available on the ferry and postings at trailheads. Some popular spots are Gåsevik, a small secluded bay that can be reached through a path in the lower fields (turn right by the red barn). Another less accessible but very nice hike is through the beech forest immediately south of the bridge to Skaftö. Take bus 845 in the direction of Uddevalla and get off at Lunnevik immediately before the bridge. There you can follow the signs for röd skogarna. If you follow the path all the way to the water, there is an old hand-drawn horse ferry. If you set out on your own, make sure you have some water and snacks and let someone know where you’re going.

## Scientific Program

### Monday April 25th

1800-2200	Arrival, registration and reception at the Sven Lovén Centre for Marine Sciences – pizza dinner
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### Tuesday April 26th

0730-0830	Breakfast
0900-0930	Introduction and Orientation (conference organizers)
	<b>Morning session: Sexual Selection &amp; Mating Systems</b> Chair: Kenyon Mobley
0930-1030	Anders Berglund, Uppsala University. "Sexual selection in pipefish"
1030-1100	Coffee break/fika
1100-1120	Onno Diekmann, Universidade Algarve. "The genetic mating system of the black striped pipefish <i>Syngnathus abaster</i> "
1120-1140	Tonje Aronsen, Norwegian University of Science and Technology. "The effects of density and operational sex ratio on the genetic mating system of the broad-nosed pipefish ( <i>Syngnathus typhle</i> )"
1140-1200	Jorge Palma, Universidade Algarve. "Courtship behavior and sex ratio effect on the reproductive behavior of the long snout seahorse, <i>Hippocampus guttulatus</i> "
1200-1220	J Pedro Andrade. Universidade Algarve. "Potential reproductive rates in male and female <i>Hippocampus guttulatus</i> , with a note on the occurrence of female bigamy"
1230-1330	Lunch
	<b>Afternoon session: Sexual Selection &amp; Mating Systems</b> Chair: Anders Berglund
1330-1350	Kimberly Paczolt, Texas A&M University. "Tradeoffs between growth and reproduction in the Gulf pipefish"
1350-1410	Gry Sagebakken, Göteborg University. "Factors affecting embryo survival in the brood pouch of <i>Syngnathus typhle</i> "
1410-1430	Gunilla Rosenqvist, Norwegian University of Science and Technology. "Sexual signals and mating patterns in Syngnathidae"
1430-1450	Kenyon Mobley, Umeå University. "Temporal patterns of sexual selection in broad-nosed pipefish, <i>Syngnathus typhle</i> "
1450-1520	Coffee break/fika
1520-1620	Charlotta Kvarnemo, Göteborg University. "Sexual selection in a monogamous seahorse"
1630-1800	<b>Poster session/reception</b>
1800-2000	Dinner

### Wednesday April 27th

0730-0830	Breakfast
	<b>Morning session: Evolution, Sexual Selection and Hormones</b> Chair: Thorsten Reusch
0900-1000	Adam Jones, Texas A&M University. "The past, present and future of mating system and sexual selection research in syngnathid fishes"
1000-1020	Charlyn Partridge, Washington University. "The effects of environmental estrogen on mating dynamics and gene expression in a sex-role reversed pipefish"
1020-1040	Emily Rose, Texas A&M University. "Effects of synthetic estrogen EE2 on sexual selection in Gulf pipefish."

1040-1110	Coffee break/fika
1110-1130	Sunny Scobell, Texas A&M University. "Hormonal mediation of female competition and male pregnancy in syngnathids"
1130-1230	Tony Wilson, Zurich Natural History Museum. "The evolutionary origins of syngnathid fishes"
1230-1330	Lunch
1330-1800	Free time/Excursions (hiking, ferry to Lysekil, etc)
1800-2000	Dinner
2000-2200	Workshop: IUCN red listing for syngnathids. Chair: Amanda Vincent

### **Thursday April 28th**

0730-0830	Breakfast
	<b>Morning session: Physiology &amp; Hormones</b> Chair: Charlyn Partridge
0900-1000	Nuno Monteiro, University of Porto. "There is a time and place for everything"
1000-1020	Ines Braga Goncalves, Göteborg University. "Embryo oxygenation in pipefish brood pouches: novel insights"
1020-1040	Jorge Palma, Universidade do Algarve. "Size of prey ingested by long snout seahorse, <i>Hippocampus guttulatus</i> . Are <i>Hippocampus spp.</i> gape-limited predators?"
1040-1110	Coffee break/fika
1110-1130	Thorsten Reusch, Leibniz Institute for Marine Sciences, Kiel. "A pipefish with a strange immune system? Transcriptomic insights into the immunogenetics of <i>Syngnathus typhle</i> "
1130-1150	Angela Bahr, University of Zurich. "Does sexual selection act on MHC genes in sex-role reversed species? Insights from the potbellied seahorse ( <i>Hippocampus abdominalis</i> )"
1150-1210	Olivia Roth, Leibniz Institute for Marine Sciences, Kiel. "Bateman's principle revisited: sex-specific immune dimorphism in a sex-role reversed pipefish"
1210-1230	Susanne Landis, Leibniz Institute for Marine Sciences, Kiel. "Who will win the hot water race?"
1230-1330	Lunch
	<b>Afternoon Session: Conservation</b> Chair: Lotta Kvarnemo
1330-1430	Amanda Vincent, Project Seahorse/University of British Columbia. "Current state of seahorse trade globally"
1430-1450	Mohammad Shokri, Shahid Beheshti University. "The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds"
1450-1520	Coffee break/Fika
1520-1540	Lucy Woodall, Project Seahorse/University of Stirling. "The conservation genetics of the European long-snouted seahorse, <i>Hippocampus guttulatus</i> "
1540-1600	Adam Lim, University of Malaya. "Diversity of syngnathid fishes in Malaysia with implications for conservation"
1600-1700	Sara Lourie, McGill University. "Taking conservation action in the face of taxonomic confusion: seahorses as a case study"
1830-2100	Banquet Dinner
2100-	Disco Pub

**Friday April 29th**

0730-0830	Breakfast
	<b>Morning session: Conservation &amp; Climate Change</b> Chair: Gunilla Rosenqvist
0900-1000	Ingrid Ahnesjö, Uppsala University. "Time perspectives on paternal pipefish pregnancies"
1000-1020	Miguel Correia, Project Seahorse/ Universidade Algarve. "Human influences on seahorse populations in the Ria Formosa lagoon, south Portugal"
1020-1040	Irecê Rosa, Universidade Federal da Paraíba. "Conservation of seahorses ( <i>Hippocampus</i> spp.) in Brazil"
1040-1110	Coffee break/fika
1110-1130	Mariana Padron, California Academy of Sciences. "Distinct phylogeographic patterns of sympatric west Atlantic exploited seahorses"
1130-1150	Bram Couperus, IMARES, Netherlands. "The snake pipefish: a coastal and truly oceanic species"
1150-1210	Josefin Sundin, Uppsala University. "Behavioral responses to a changing environment"
1210-1230	Concluding Remarks (Conference Organizers)
1230-1330	Lunch
	**End**





## Abstracts – Oral

Abstracts are listed alphabetically by **presenting author**

## Time perspectives on paternal pipefish pregnancies

**Ingrid Ahnesjö**

*Dept. Ecology & Genetics / Animal Ecology, Uppsala University, Norbyv. 18D, SE-752 36 Uppsala, Sweden*

Historically, one of the first publications ascribing the pouch bearing of a pipefish to the male was done by Ekström (1831). Now we know that in all Syngnathidae species females transfer eggs to the male, who will fertilize and brood them, often for prolonged periods, and the form of care varies among species. In the broad-nosed pipefish, *Syngnathus typhle*, we have a multifaceted understanding (but far from complete) of their reproductive ecology, full of interactions and consequences of the male pregnancy. The predominant female competition for male mating partners is a consequence of the time consuming male brood care. Furthermore, the timing and quality of maternal egg provisioning, timing and quality of paternal care provisioning (nutrients, oxygen etc.) interact and influence offspring fitness. There may be competition, conflicts, constraints and compensations over time and resources. What reproductive strategies in the two sexes are selected and when? This will be an overview over time and about time in the reproductive world of a pipefish.

## Potential reproductive rates in male and female *Hippocampus guttulatus*, with a note on the occurrence of female bigamy

J. Pedro Andrade, A. I. Fialho, Jorge Palma

Centre for Marine Sciences, University of the Algarve, 8005-139 Faro, Portugal

In this study, the potential reproductive rate of male and female *Hippocampus guttulatus* has been estimated under controlled conditions. The duration of “time in” was 7.8 and 5.1 days for females and males, respectively. The reproductive “time out” was 27 days for females and 33 days for males.

Males showed a significantly shorter latency to mate than females (males  $5.1 \pm 0.9$  days, females  $7.8 \pm 1.1$  days; Mann–Whitney U;  $P=0.0051$ ). Time from copulation to subsequent copulation was  $32 \pm 8.7$  days for males and  $27 \pm 10.3$  days for females; Mann–Whitney U;  $P=0.5714$ ).

This is the first record of a consistent female bigamy in a seahorse species: in 40% of the trials in which a male and female successfully copulated and the male gave birth, the females were observed to copulate with another male, become pregnant and gave birth to viable offspring. Thus, over the course of one breeding season, females could potentially produce 8.3% more offspring than males:  $379.\text{female}^{-1}$  vs  $314.\text{male}^{-1}$ .

Our results are in contrast with observations of the potential reproductive rates and reproductive “time out” for other *Hippocampus* species, as *H. zosterae*. This might indicate that *H. guttulatus* females would compete more intensively than males for access to mates. These results are discussed in light of female bigamy observed during the course of the experiments.

## **The effects of density and OSR on the genetic mating system of the broad nosed pipefish (*Syngnathus typhle*)**

**Tonje Aronsen**<sup>1</sup>, Kenyon Mobley<sup>1,2</sup>, Anders Berglund<sup>3</sup>, Gunilla Rosenqvist<sup>1</sup>

<sup>1</sup>*Department of Biology, Norwegian University of Science and Technology (NTNU), N-7491 Trondheim, Norway,* <sup>2</sup>*Department of Ecology and Environmental Science, Umeå University, 90187 Umeå, Sweden,* <sup>3</sup>*Department of Ecology and Genetics/Animal Ecology, Uppsala University, Norbyvägen 18 D, SE 752 36 Uppsala, Sweden*

The operational sex ratio (OSR) and population density might influence the opportunity for and the strength of sexual selection. Density effects have been understudied in behavioural ecology, but it has been shown that population density can have a profound effect on the mating system and the strength of sexual selection indicating that selection pressures related to sexual selection might differ at different densities.

We have performed a replicated experimental study with breeding populations of the broad nosed pipefish (*Syngnathus typhle*), at different OSR's and densities. We then used three microsatellite markers to resolve the genetic mating system.

The goal of this experiment was to investigate whether the OSR and also the density of potential partners and competitors can influence the genetic mating system and the variation in reproductive success.

## **Does sexual selection act on MHC genes in sex-role reversed species? Insights from the potbellied seahorse (*Hippocampus abdominalis*)**

**Angela Bahr**, Anthony B. Wilson

*University of Zurich, Institute of Evolutionary Biology and Environmental Studies, Zurich, Switzerland*

Both natural and sexual selection are thought to influence the genetic diversity of major histocompatibility (MHC/MH) loci, an integral part of the vertebrate adaptive immune system. In teleost fishes, the importance of MH-based mate choice has been demonstrated in conventional, female-based mating systems, but it remains unclear whether these molecules play similar roles in sex-role reversed species. Sex-related differences in the detection and use of MH-odor cues are expected to influence the intensity of sexual selection in such species, potentially leading to major differences in the pattern of MH variation.

We are investigating the role of MH class II beta-chain genes during mate choice in the sex-role reversed seahorse *Hippocampus abdominalis*. The results of targeted gene sequencing are congruent with transcriptome screening, indicating the existence of a single expressed MHII $\beta$  gene in this species. The variability of the seahorse MHII $\beta$  antigen-binding region is high, comparable to that found in species with conventional sex roles and a single MHII $\beta$  gene.

Olfactory-based experiments have been used to investigate the role of MHII $\beta$  during mate choice in *H. abdominalis*. Preliminary analyses suggest that while females show a preference for MH-dissimilar males, males show no MH-based preferences. Free mate choice trials are currently being used to determine whether these preferences are still detectable under semi-natural conditions.

## Sexual selection in pipefish

**Anders Berglund**

<sup>1</sup>*Department of Ecology and Genetics/Animal Ecology, Uppsala University, Norbyvägen 18 D, SE 752 36 Uppsala, Sweden*

In a small but interesting minority of all animal species, sex roles are reversed: females ready to mate are in excess, and therefore they compete for males. In the sex role-reversed pipefish *Syngnathus typhle* males devote more time, but not more energy, than females to offspring production. The long male pregnancy (offspring are brooded and nourished by males on their bodies) lower their potential reproductive rate below that of females. As females are faster reproducers the operational sex ratio became skewed toward an excess of females. Thus, basically males are more choosy and females more competitive, but in addition males may also compete with other males, and females may to some degree be choosy. Moreover, females exhibit a stronger positive association between number of mates (as determined by microsatellite analysis) and fertility than do males, so the relationship between mating success and number of progeny, as characterized by the Bateman gradient, affects the strength and direction of sexual selection.

When given a choice, *S. typhle* males prefer large over small females, parasite-free over parasitized females, and ornamented over non-ornamented females. Larger females also dominate smaller females, as a result gaining an additional reproductive advantage. A temporary color ornament in *S. typhle* females is used in female-female competition, attracts males, is independent of nutritional level, and honestly predicts fecundity and mating success in females. Ornament design is not arbitrary: it facilitates the detection of size differences between females.

The choice of partners confers fitness benefits to the choosing individual, whether male or female. Offspring from matings with preferred partners are superior at escaping predators, whether males or females perform the choice. Moreover, experience modulates mate choice, and differently in males of different status: high status males take risks to choose the best females, whereas low status males play it safe and make choices that minimize competition from other males.

# Human influences on seahorse populations in the Ria Formosa lagoon, south Portugal

Miguel Correia<sup>1,2</sup>, Heather Koldewey<sup>2</sup>, José Pedro Andrade<sup>1</sup>

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Changes in seahorse numbers have commonly been ascribed to overfishing but other forms of human impact may be important, too. We here examine population dynamics of two species of seahorse (*Hippocampus guttulatus* and *Hippocampus hippocampus*) in the Ria Formosa lagoon where there are no targeted fisheries. Recent studies have suggested that the number of seahorses, once very abundant, have declined precipitously. We conducted a more comprehensive survey throughout the lagoon (January to September 2010) to establish the extent of declines. These surveys found seahorse densities averaged  $0.058 \pm 0.025$  seahorse.m<sup>-2</sup> for *H. guttulatus* and  $0.014 \pm 0.009$  seahorse.m<sup>-2</sup> for *H. hippocampus* and maximum density was 0.19 and 0.07 seahorse.m<sup>-2</sup>, respectively. These compare with previous reports of maximum densities of 0.51 and 0.07 for the two species. Pregnant males were observed from May to August. The absence of local boat traffic – banned by local authorities – was correlated with a significant increase in seahorse numbers in one site ( $0.05 \pm 0.02$  and  $0.16 \pm 0.02$  seahorse.m<sup>-2</sup>). Our work suggests that boat traffic and other anthropogenic variables may be important in explaining changes in seahorse population numbers. We will discuss how the distribution of seahorses and understanding their population dynamics is being used to develop a conservation management plan for the lagoon.

## The snake pipefish: a coastal and a truly oceanic species

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Beginning 2004 a massive invasion of snake pipefish, *Entelurus aequoreus*, into waters of the northeast European shelf was observed. This invasion peaked in 2007 and 2008 reaching far up North into the Barents Sea and up to Spitsbergen. The most puzzling observation was that almost all of these occurrences were pelagic, often far offshore while this species was at the time primarily considered as being coastal.

This study presents the full extent of the appearance of the snake pipefish on the northeast European shelf. Starting with the appearance from the open ocean and outburst along the full northern European shelf and North Sea. Re-examination of plankton and bottom trawl survey data west of the British Isles revealed that first offshore findings occurred in the western areas with subsequent spreading of the species to the North and East. These findings suggest, that snake pipefish were advected from the West, thus from the open ocean. Since snake pipefish is not known from the Western Atlantic margin, we conclude that a population above the Mid Atlantic Ridge might exist. We will discuss possible explanations on why this sudden invasion occurred.



# The genetic mating system of the Black Striped pipefish *Syngnathus abaster*

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Pipefish and seahorses (family Syngnathidae) are peculiar creatures because in this group of animals the males provide all parental care. In the breeding season females transfer unfertilized eggs to the male's ventral surface where they are fertilized. Brooding structures in males range from fully enclosed brooding pouch to the simple "gluing" of eggs to the male's external ventral surface. In the pipefish *Syngnathus abaster*, eggs are incubated in a pouch consisting of two ventral folds where fertilization occurs. This internal fertilization ensures paternity of the brooding male.

Observations in *S. abaster* refer to a polygynandrous mating system with females mating with different males and males receiving eggs from different females. However this has never been confirmed genetically. With the development of microsatellites for this species we were able to perform parentage analysis to answer the following questions: 1) do male *S. abaster* mate with more than one female. 2) do female *S. abaster* spread the risk by depositing eggs in multiple males (bet hedging) .

Results presented here confirm multiple maternity in *S. abaster* and there is also evidence that females mate with more than one male.

## Embryo oxygenation in pipefish brood pouches: novel insights

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In many syngnathids males provide protection, oxygenation, nourishment and osmoregulation to the developing embryos. In an experimental study, we investigated how embryo size and survival are affected by ambient hypoxia in four pipefish species. Two species, *Entelurus aequoreus* and *Nerophis ophidion*, have simple ventral attachment of eggs onto the male trunk and the others (*Syngnathus typhle* and *S. rostellatus*) have fully enclosed brood pouches on the tails. All species showed lower embryo survival under hypoxia, however, species with brood pouches suffered greater embryo mortality compared to species without pouches, irrespective of oxygen treatment. In addition, we assessed to what extent *S. typhle* males are able to maintain within-pouch oxygen levels when brooding in high or low ambient oxygen. In both treatments, oxygen levels were lower inside the pouch than in the surrounding water. Internal pouch oxygen levels decreased throughout the brooding period, highlighting a greater offspring demand for oxygen during development or a decreasing paternal ability to maintain within-pouch oxygen levels. Importantly, embryo size was positively correlated with oxygen availability and larger males had higher within-pouch oxygen levels than smaller males. In conclusion, our results reject the hypothesis that syngnathid brood pouches are well-oxygenated structures that promote the evolution of larger eggs, as we found no significant benefits of brood pouches in terms of embryo survival and size under hypoxia.

# **The past, present and future of mating system and sexual selection research in syngnathid fishes**

**Adam G. Jones**

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Pipefishes and seahorses have been at the forefront of the microsatellite revolution in behavioral ecology since it began about 15 years ago. Microsatellite-based studies of parentage in these fishes have led to important general insights into mating system evolution and the interpretation of patterns of mating in light of sexual selection theory. The impressive diversity of syngnathid mating systems, which range from strict monogamy to perfect polyandry, sets the stage for key comparative studies of mating system evolution in a phylogenetic context. In addition, newer work, which shows that syngnathid mating systems vary both temporally and spatially within species, identifies another potential avenue for the elucidation of factors shaping mating systems. The study of mating systems in natural populations provides some statistical challenges, but future work involving syngnathid fishes has the potential to make fundamental contributions to behavioral ecology and evolutionary biology. The most obvious areas of need for additional research include better characterization of mating system variation within species, investigations of the causes of mating system variation, and better coverage of species and genera with respect to mating system data.

## **Sexual selection in a monogamous seahorse**

**Kvarnemo, Charlotta**

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Sexual selection is typically expected to be weak in monogamously breeding animals. Yet, when both sexes vary in mate quality, such variation can generate mutual mate choice, assortative pair-formations, and ultimately inter-sexual selection on both sexes. In addition, biased adult sex ratios may also cause marked sexual selection, when low quality individuals of the sex in excess find no mates at all. In seahorses body size is likely to correlate with mate quality in both sexes. In a field based genetic study of the monogamous Western Australian seahorse, both opportunity for sexual selection and sexual selection differentials indicate that females are under stronger sexual selection than males, as many females remained unmated and mated females were larger than unmated ones, while there was no size difference among mated and unmated males. This result, generated by a combination of size-assortative pair formations and female biased adult sex ratios, shows us that sexual selection is not necessarily weak in monogamous species. To investigate which sex contributes most to the reproductive success of a pair by being large, we carried out a cage experiment, with size-matched and mismatched pairs. However, the results from this experiment were unfortunately inconclusive. Finally, whether the size-assortative pairs result from one or both sexes being choosy or competitive still remain to be investigated.

## Who will win the hot water race?

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Selection may favour altered combinations of host and parasite genotypes in different environments that can shape the geographic distribution of genetic diversity and patterns of local adaptation. But what happens when a stable environment gets warmer due to climate change? Can an extreme event of climate change disrupt locally adapted host-parasite combination and create new hot and cold spots of coevolution?

To determine how different climates and spatial divergence shapes local adaptation of host and parasite, we analysed the population structure and genetic variability in four populations of our model system, the pipefish *Syngnathus typhle* and the trematode *Cryptocotyle lingua* along a north-south gradient from Sweden and Denmark over Germany to Italy. We used thirteen microsatellite, three mitochondrial markers and a marker of the major histocompatibility complex I (MHC I). Doing so, we could disentangle positive selection (MHC I marker) from neutral selection (microsatellites and mitochondrial markers) and determine ancient versus recent evolution.

In addition, we experimentally investigated how a heat wave as an extreme event of global change alters the immune response of the host (*S. typhle*) and its interaction with sympatric or allopatric parasites (*Cryptocotyle lingua*). Whereas parasites showed a clear pattern of local adaptation, the heat-wave led to drastic changes in the immune response of pipefish. Taken together, our data suggest that host-parasite dynamics may shift under global warming.

## **Taking conservation action in the face of taxonomic confusion: seahorses as a case study**

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A clear taxonomy is vital for conservation research, legislation and management. It enables unambiguous communication among researchers, legislators, stakeholders, and conservationists; it provides a framework within which information can be gathered, stored and retrieved; and it allows decisions to be made based on closely related species in cases where specific information is lacking. The reality, however, is that taxonomy is an active field of research and clarity is not always readily achievable. This is particularly true in cases where our current understanding of species distributions and population connections is limited, and where the effect of the environment on morphological variation is poorly understood. Seahorses are such a case. Despite a long taxonomic history dating back to Linnaeus, it was not until 1999 that the first attempt at a global revision of the genus was published. Subsequent research has focused on genetic patterns, and regional revisions. Twenty-one new species have been described in the past ten years, and a number of other, previously synonymised names have been resurrected. The basis upon which these decisions have been made, however, is not always clear, and in some cases the evidence is contradictory, resulting in dissent among researchers. Given the conservation concerns for these unusual fishes, it is time that these disparate views be brought together into a coherent overview, and that appropriate tools be developed to meet the needs of conservation practitioners, bearing in mind the realities of the multi-scale nature of biodiversity.

## **Temporal patterns of sexual selection in broad-nosed pipefish, *Syngnathus typhle***

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In 2005 I conducted an intensive survey of a single population of *Syngnathus typhle* in the Gullmarsfjord and returned to the same location in 2006. I used microsatellite markers combined with parentage analysis and genetic mark-recapture techniques to estimate population size and the genetic mating system. The study reveals some fascinating insights. First, sexual selection on males and females varies between years in the same site. Second, recapture rates of males and females from 2005 are different suggesting that females are more mobile than males. Third, the strength of sexual selection as measured by various variance indices such as Bateman's principles are not related to the operational sex ratio encountered at the time of collection. Fourth, there is no evidence of size assortative mating encountered in any of the collections. The last two revelations demonstrate that there is a disconnect between what is traditionally known about individual-based behavioral decisions and in situ population-level interactions. Thus, in order to gain a better understanding of how sexual selection operates in the wild, we will need to consider how ecological parameters and temporal fluctuations in the genetic mating system affect the outcome of sexual selection.

## There is a time and place for everything

**Nuno Montiero**

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If there really is a time and place for everything, then everything changes according to time and place. However awkward this low-quality axiom may sound, if it holds any truth, then it nicely depicts many of the observations that I have conducted, during the past few years, regarding pipefish life history. By mainly addressing the results gathered on two very distinct species, the worm pipefish, *Nerophis lumbriciformis*, and the black striped pipefish, *Syngnathus abaster*, I will try to illustrate the dynamic nature of reproduction, either through space or time, by looking at variables such as migration schedules, age at first reproduction, frequency of reproduction, number of eggs, and parental investment in offspring. I will also speak on how syngnathids can be viewed as potentially accurate indicators of environmental stress, namely by signaling, even from earliest stages of their ontogenic development, the presence of endocrine disruptors. Some of the compiled results, from alterations in reproductive patterns to disruptions in recruitment events, are especially important if we take into account that most of the studied syngnathid populations have a coastal distribution, where xenobiotics are commonly present. Thus, once again, time (sampling date) and space (sampling location) can play a decisive role as, due to anthropogenic-derived factors, some of the usually measured variables have the potential to bias our interpretations.



## Diversity of syngnathid fishes in Malaysia with implications for conservation

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This study is the first comprehensive documentation on the diversity of fishes from the family Syngnathidae within Malaysia and its adjacent waters. Records of syngnathids from unpublished data, reviewed literatures, museum collections and results from the present study were examined and collated. High species richness with a total of 51 species encompassing all subfamilies of the Syngnathidae, the Hippocampinae, Doryrhamphinae, Syngnathinae and Solegnathinae, is recorded from Malaysian waters. Five species, *Hippocampus severnsi*, *Kyonemichthys rumengani*, *Notiocampus ruber*, *Siokunichthys bentuviai* and *Syngnathus pelagicus* are species previously not believed to be distributed within the Malaysian region. Highest species richness was recorded around the Semporna Islands, Sabah. Habitats where syngnathids were recorded include marine macroalgal beds, coral reefs, coastal, estuaries, rivers and streams. Syngnathid fishes appear to be cryptic, live in low densities and display a patchy distribution. The major threats to syngnathid conservation in Malaysia include purported exploitations, by-catches, habitat destructions and degradations, recreational threats and pollutions.

## **Tradeoffs between growth and reproduction in the Gulf pipefish**

**Kimberly A Paczolt**, Adam G Jones

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Male pregnancy provides a unique opportunity to investigate post-copulatory sexual selection in sex-role reversed taxa. Our previous results, in the Gulf pipefish, show that pregnant male may use different resource investment strategies depending on the perceived quality of his offspring as well as the quality of his previous broods, a pattern consistent with cryptic male choice. Here, we investigate the interaction between resource availability, growth, and reproduction. We ask the question, does resource availability affect the survivorship of broods from attractive and unattractive mates? We show that males under low food conditions have lower levels of offspring survivorship than males under high food conditions. Furthermore, these males employ tradeoffs between growth and reproduction, and this tradeoff is biased in favor of broods from attractive mates.

## Distinct phylogeographic patterns of sympatric west Atlantic exploited seahorses

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Major issues of conservation relevance, ranging from assessments of spatial and temporal population structure to analysis of phylogenetic relationships, can be addressed with molecular genetic approaches. To provide a better understanding of population connectivity and the identification of potentially cryptic evolutionary lineages in two sympatric seahorse species from the Caribbean and western Atlantic Ocean, *Hippocampus erectus* and *Hippocampus reidi*, we collected 115 specimens from six different populations and analyzed both mitochondrial and nuclear DNA (cytochrome *b*, mitochondrial control region, and 5 polymorphic microsatellite loci). The results obtained from mtDNA, indicate that *H. erectus* and *H. reidi* exhibit different evolutionary histories, and microsatellite data is being collected for comparison.  $F_{ST}$  values indicate that populations of *H. erectus* are not highly structured. On the contrary, populations of *H. reidi* are highly structured, with a population from Brazil being almost completely isolated. Reciprocal monophyly for the Brazilian haplotypes, and their relationship to its sister species, *H. algiricus*, even suggest a putative new species for that region. Our results suggest that the patterns and processes that determine and maintain species distributions, and than can help inform conservation decisions, are distinct between these two species. The implications of these patterns for conservation may include the identification of management units and distinct phylogeographical breaks, perhaps indicative of dispersal differences across species or different responses to selection, and new ecological hypotheses about the mechanisms that possibly maintain their current distributions

## **Size of prey ingested by long snout seahorse, *Hippocampus guttulatus*. Are *Hippocampus* spp. gape-limited predators?**

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This investigation examined *Hippocampus guttulatus* feeding strategy and possible gape limitations using the Atlantic ditch shrimp (*Palaemonetes varians*) as a model. Three significantly different length sizes (13-17mm or S1, 18-22mm or S2 and 23-26mm or S3) of the shrimp were fed to two significantly different height classes (12-13cm or C1 and 14-15cm or C2) of *H. guttulatus*. The mean thorax sizes of shrimp in the size classes of the experiment were 7.55mm, 11.66mm and 17.08mm for S1, S2 and S3 shrimp, respectively. The minimum mouth area (gape) recorded was 4.34mm<sup>2</sup>, while the maximum reached 13.99mm<sup>2</sup>. The feeding strategy and amount of each class of consumed prey was observed, recorded and correlated with morphological characteristic of both prey and predator. All three shrimp classes were eaten by the smaller sized seahorses (C1). S1 were eaten whole in a sucking motion; S2 were killed and then eaten whole; while S3 were attacked until dead and then eaten in parts. Feeding rate in C1 were higher when fed S1 (4.32±0.54 %bw.d<sup>-1</sup>), when compared to the remaining prey size classes (3.93±0.62 and 3.67±0.58 %bw.d<sup>-1</sup> for S2 and S3, respectively). These results showed that the ingestion of prey by *H. guttulatus* is not restricted to the prey size or seahorse mouth gape which demonstrates a higher plasticity in the *Hippocampus* species prey size range.

# Courtship behaviour and sex ratio effect on the reproductive behaviour of the long snout seahorse, *Hippocampus guttulatus*

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This study characterizes the standard courtship behaviour of the long snout seahorse, *Hippocampus guttulatus*, and the sex ratio effect. Irrespectively of the analysed gender proportions (1♀:1♂, 1♂:3♀ and 1♀:3♂), 86% of the daily greetings were observed during the morning period and 14% in the afternoon with the copula occurring always in the afternoon. The participation of each sex in the courtship behaviour did not changed significantly ( $P>0.05$ ) in dependence to the number or proportion of sexes, with males always assuming a more active role in the courtship behaviour. Time periods expended in courtship activities did not changed significantly ( $p>0.05$ ) lasting,  $161\pm 106$ sec.,  $173\pm 120$ sec. and  $195\pm 148$ sec. in three fish proportions (1♀:1♂, 1♂:3♀, 1♀:3♂) but a significant reduction ( $p>0.05$ ) in the resting time between courtship interactions, (respectively, as above),  $489\pm 544$ sec.,  $318\pm 298$ sec. and  $272\pm 286$ sec. was observed. The duration of the first breeding cycle was longer ( $39\pm 14.8$ days,  $39.5\pm 14$   $42.1\pm 13.6$ ) than the following cycles ( $21.2\pm 7.1$  days,  $21.2\pm 8.5$ ,  $21.3\pm 9.2$ ) as the courtship behaviour was only observed in the first cycle. Parturition was a discontinuous procedure lasting  $1.7\pm 0.9$  days and the following pregnancy initiate after one day of parturition recovery. In comparison to the majority of other *Hippocampus* species, this study identified singularities in the *H. guttulatus* courtship behaviour such as the extension of the daily greetings and copula in to the afternoon period. It was also observed that an increase in the sex proportion boosts the sex competition which leads to an acceleration of the reproductive process of *H. guttulatus*.

# The effects of an environmental estrogen on mating dynamics and gene expression in a sex-role reversed pipefish

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Environmental pollutants have been shown to affect normal reproductive mechanisms in a number of different species. While many studies have addressed the impact these compounds have on primary sexual characters (i.e., gonads and ovaries), few studies have examined how these compounds may affect secondary sexual traits and in turn pre-copulatory sexual selection mechanisms. In this study, we examined how exposure to a synthetic estrogen, 17 $\alpha$ -ethinylestradiol (EE2), impacted mating dynamics and gene expression in a sex-role reversed pipefish. Our results showed that only 10 days of exposure to low levels of EE2 resulted in adult male pipefish developing female-like secondary sexual traits. While these males were capable of reproduction, females discriminated against exposed males in mate choice trials. In addition, exposure to high levels of EE2 resulted in decreased male mating success when compared with control males. In natural populations, this type of discrimination would reduce male mating opportunities, thus potentially reducing their long-term reproductive success. In addition, gene expression analyses were also conducted on abdominal and pouch tissue from exposed and non-exposed males. Based on these data, we will discuss potential genes within these tissues that are affected by exposure to EE2.

## **A pipefish with a strange immune system? - transcriptomic insights into the immunogenetics of *Syngnathus typhle***

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Within the pipefish group at IFM-GEOMAR Kiel, we have recently begun to study the host parasite interactions among broad-nosed pipefish and their bacterial pathogens and metazoan parasites. As one starting point for characterizing the genetic basis of the pipefish immune defence, classical genes of the major histocompatibility complex (MHC) were indentified. MHC class I genes were successfully isolated and genotyped in replicated individuals and populations. Their polymorphism revealed the hallmark of diversifying (balancing) selection as an elevated ratio of silent versus amino-acid changing substitutions (dN /dS) and of allelic divergence between geographically separated locations. In order to fully cover most immune relevant genes in *S. typhle*, we then produced a normalized EST library obtained by 454 pyrosequencing that comprises 400,000 reads of an average of 450 bp length from immune challenged fish and various tissues. The resulting ~8000 putative unigenes will serve as a valuable genetic resource to the entire pipefish community. Interestingly, several genes related to the MHC class II pathway were absent from our data, such as the T-cell receptor and the MHC class II genes themselves.

## Conservation of seahorses (*Hippocampus* spp.) in Brazil

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Treasured as symbols of the seas, seahorses (*Hippocampus* spp.) are recognized as (1) an important flagship species to marine conservation; (2) globally threatened by overexploitation and habitat destruction; (3) fishes with high economic value and marketability; (4) particularly vulnerable to heavy exploitation due to some of their life-history traits, which include low potential reproductive rate, monogamy, small home range, and limited swimming abilities. An integration of the aforementioned aspects requires, among other aspects, the development of strategies encompassing both ecological and socioeconomic perspectives. Brazil is recognized as one of the main suppliers of seahorses for the aquarium trade, and as the only major exporter and the main domestic consumer of live seahorses in Latin America, thus being a relevant case study for discussing conservation of seahorses and their habitats. This study presents an overview of seahorse research being carried out in Brazil, and discusses conservation initiatives and gaps in knowledge.



# Effects of synthetic estrogen (EE2) on sexual selection in Gulf pipefish

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Pipefish are excellent evolutionary models because of their unique morphology, such as a male brood pouch structure and male parental care. We investigated the effects of the synthetic estrogen 17 $\alpha$ -ethinylestradiol, EE2, on pre and post-copulatory sexual selection in gulf pipefish. Gulf pipefish, *Syngnathus scovelli*, are sex-role reversed fishes, which boast strong sexual dimorphism and secondary sex characteristics such as iridescent bars on sexually mature females. Endocrine disruptors such as EE2 have been shown to alter gene expression and cause morphological feminization in males. Enclosure tanks housed eight male and eight female gulf pipefish exposing half the treatments to 2ng/L EE2. By comparing these treatments we determined the effects of EE2 on sexual selection in this species. We assessed pre and post-copulatory sexual selection by the number of eggs transferred, survivorship of the embryos, and male preference by assigning parentage.

## Sexual signals and mating patterns in Syngnathidae

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Male pregnancy in the family Syngnathidae (pipefishes, seahorses and seadragons) predisposes males to limit female reproductive success and sexual selection may then operate more strongly on females and female sexual signals may evolve (“sex role reversal”). A bewildering array of female signals have evolved in Syngnathids, e.g. skin folds, large body size, colouration, markings on the body and elaborate courtship. These female sexual signals do not seem quantitatively or qualitatively different from those that evolve in males in species with conventional sex roles where males provide females or offspring with direct benefits. In several syngnathid species also males evolve ornaments, females are choosy in addition to being competitive, and males compete as well as chose partners. Thus, sex roles form a continuum, spanning from conventional to reversed within this group of fishes. Cases are here presented suggesting that stronger sexual selection on females may be most extreme in species showing classical polyandry (one male mates with several females, such as many species where males brood their eggs on the trunk), intermediate in polygynandrous species (males and females both mate multiply, as in many species where males brood their eggs on the tail), and least extreme, even exhibiting conventional sex roles, in monogamous species (one male mates solely with one female, as in many seahorses and tropical pipefish). At the same time caution is needed before unanimously establishing this pattern: first, the connection between mating patterns, strength of sexual selection, sex roles and ornament expression is far from simple and straightforward, and second, knowledge of the actual morphology, ecology and behavior of most syngnathid species is scanty: basically only a few *Nerophis*, *Syngnathus* and *Hippocampus* species have been studied in any detail. It is known, however, that this group of fish exhibits a remarkable variation in sex roles and ornamentation, making them an ideal group for the study of mating patterns, sexual selection and sexually selected signals.

## Bateman's principle revisited: sex-specific immune dimorphism in a sex-role reversed pipefish

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In diverse animal species, from insects to mammals, females have a more efficient immune defence than males. Bateman's principle posits that males maximize their fitness by increasing mating frequency whilst females gain fitness benefits by maximizing their lifespan, which in turn requires a more efficient immune system. Because in most extant animals, female function (i.e. provisioning of eggs) and higher parental investment are correlated within the female, sex-role reversed species are required to demonstrate that immune dimorphism depends on life-history and not on sex per se. Our model species is the broad-nosed pipefish *Syngnathus typhle*, where males brood the eggs in a paternal brood pouch and thus, invest more into reproduction than females. Concordant to an extension of Bateman's principle, we found males to have a more efficient immune response both in field data from four populations, and also in an experiment under controlled laboratory conditions. This applied to four different measures of immune competence of the innate as well as from the adaptive immune system. We further determined the specificity of mounting immune responses after a fully factorial primary and secondary exposure to a common marine pathogen *Vibrio spp* and found males to not only have a more efficient but even a more specific immune defence than females.

## **Factors affecting embryo survival in the brood pouch of *Syngnathus typhle***

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Parental care may contain several aspects of care or lack thereof. Brood reduction is generally thought to imply a termination of care and loss of fitness for the caring parent. However, there may be a gain of brood reduction. Brood reduction may have many causes and effects on the population, and the fitness effects of the caring parent may vary. In the broad-nosed pipefish (*Syngnathus typhle*) the caring male is actually able to absorb nutrition from the embryos through his pseudo placenta/brood pouch. This species is sex-role reversed and the male care for his embryos in his brood pouch for about a month. During the brood period there is on average 20% reduction of embryo numbers during the brood period. We have performed different mating treatments on a natural population of broad-nosed pipefish to see what might affect brood reduction. Parameters we measured several characters like mating regime, the brooding male's condition and the number and size of embryos the father is caring for.

# Hormonal mediation of female competition and male pregnancy in syngnathids

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Despite a long history of fascination with sex-role reversal and male pregnancy in syngnathids, very few studies have examined the underlying hormonal mechanisms that mediate these traits. We have conducted several studies on the role of androgens in female competitive behavior, and from our recent review of the literature, we developed a model for the hormonal regulation of the male reproductive cycle in seahorses. 11-oxygenated androgens such as 11-ketotestosterone and 11 $\beta$ -hydroxyandrostenedione stimulate male sexual coloration and courtship behavior in many species of fish and are likely candidates for mediating sex-role reversed behavior in female syngnathids. In a series of studies, we determined that 11 $\beta$ -hydroxyandrostenedione is correlated with winning a short-term intrasexual competition in female Gulf pipefish, *Syngnathus scovelli*. However, injections of 11-ketotestosterone did not affect female competitive behavior. In all likelihood, androgens also regulate spermatogenesis and the development of the brood pouch prior to pregnancy in male syngnathids. Following mating, the data suggests that prolactin and corticosteroids synergistically mediate brood pouch function in seahorses. Neuropeptides such as arginine vasotocin or isotocin likely regulate parturition and may play a role in mediating reproductive behavior. The diversity of reproductive patterns exhibited by syngnathids suggests that they will provide a unique opportunity to assess how hormonal regulation of reproductive behavior and function has evolved within a teleost fish lineage.

## **The effectiveness of seahorses and pipefish (Pisces: Syngnathidae) as a flagship group to evaluate the conservation value of estuarine seagrass beds**

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Syngnathids (Pisces, Syngnathidae: seahorses and pipefish) were investigated for their use as a flagship group to evaluate the conservation value of estuarine seagrass beds in estuaries in south-east Australia. Some species of syngnathids are listed internationally as vulnerable or endangered, and they are a charismatic group of fish that attracts a high level of public support and sympathy. Syngnathids are also protected in several states of Australia. Conservation of syngnathids might provide coincidental benefits to other species that share their habitats. The effectiveness of syngnathids as a flagship group was assessed by (1) testing for correlations with other fish in species richness, density, assemblage variation, and summed irreplaceability value, and (2) determining the number of species of all other fish coincidentally captured in marine protected areas (MPAs) selected for syngnathids. The study was undertaken in a single estuary (scale: tens of square kilometres) and across multiple estuaries (scale: hundreds of square kilometres). Densities of syngnathids and other fish were correlated only at the scale of multiple estuaries. Species richness and summed irreplaceability of syngnathids and other fish were not spatially correlated. Spatial variations in assemblages of syngnathids and other fish were correlated. MPAs selected for syngnathids included more non-syngnathid species than a random selection of locations. This study provides evidence that ranking the conservation value of seagrass beds on the basis of the density and assemblage variation of syngnathids, and selecting MPAs to represent syngnathid species, will simultaneously benefit other fish. Syngnathids are therefore regarded as a useful flagship group for conservation planning.

## Behavioural responses to a changing environment

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Aquatic ecosystems are changing due to increased amounts of filamentous algae and phytoplankton. The broad-nosed, *Syngnathus typhle*, and straight-nosed, *Nerophis ophidion*, pipefish inhabits shallow eelgrass meadows, vulnerable to eutrophication. Field data shows that pipefish do not occur as frequently in eelgrass covered with filamentous algae as in clean eelgrass. The preference of juvenile and adult pregnant males was tested in an experiment, and the field observation was confirmed; *S. typhle* and *N. ophidion* strongly preferred clean eelgrass to eelgrass overgrown with filamentous algae.

Phytoplankton alters the environment of pipefish and other species inhabiting shallow coastal areas. Turbidity reduces visibility and such changes may affect animal behaviour as well as evolutionary processes that are dependent on visual stimuli. If sexual selection on visual signals is impaired, other signals, such as chemical or auditory signals might become more important. Here, we focus on the use of visual and olfactory cues in mate search, mate choice, and reproductive success in *S. typhle*. Our results show that the broad-nosed pipefish cannot locate females by the use of chemical cues alone. In mate choice there was a strong effect of reduced visibility on male mate choice behaviour, implying an environmental dependence. Thus, increased turbidity may affect processes of sexual selection through an impaired possibility to use visual signals. However, reproductive success, in terms of number of eggs received and number of mated males, did not differ between clear and turbid treatments. These results together imply complex picture on the effects of eutrophication in this species.

## Conservation and management of seahorses

**Amanda C.J. Vincent**<sup>1</sup>, Heather J. Koldewey<sup>2</sup>, Melissa Evanson<sup>1</sup>, Sarah J. Foster<sup>1</sup>

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We will analyse pressures on seahorses and explore conservation responses. The paper combines a synthesis of published literature with new data on the trade in seahorses for traditional medicine, aquarium display and curiosities. In 2002, they became the first fishes of commercial importance to be included on CITES Appendix II, requiring that all exports be limited to sustainable levels.

Key findings from CITES data for 2004-2008 are that (a) the vast majority of trade was in dried seahorses, (b) 28 species were declared in trade across all years, (c) 71 countries/jurisdictions were reported as sources and/or consumers of seahorses, and (d) trade in live seahorses has largely shifted from wild to cultured seahorses. Most traded seahorses come from trawl bycatch, although seahorses are also targeted. That said, not all changes in population abundance of syngnathids can be attributed to fishing.

A first review of the effect of habitat change on syngnathids indicates that some species may cope better than others. Syngnathid fishes can certainly be affected (lethally or sub-lethally) by physical degradation and destruction of their habitats. They may also suffer from reduced water quality arising from lower light levels or hypoxia associated with eutrophication or pollution.

The combination of pressures means that most species of syngnathid are now included in the IUCN Red List of Threatened Species or national equivalents. Allee effects may contribute to population decline in seahorses. Possible conservation measures include marine protected areas, fisheries management, select aquaculture ventures, trade regulation, improved governance (particularly) and consumer engagement.



# The evolutionary origins of syngnathid fishes

**Anthony B. Wilson**

*Institute of Evolutionary Biology and Environmental Studies, University of Zurich, Switzerland*

The past several decades have been a 'golden age' for syngnathid research, and seahorses and pipefishes are now firmly established as important models for a wide range of evolutionary questions. However, despite their significance as evolutionary and ecological model systems, phylogenetic relationships among gasterosteiform fishes remain poorly resolved, complicating efforts to understand the evolutionary origins of their exceptional morphological and behavioural diversity. Morphological and molecular analyses have helped to illuminate evolutionary relationships at the population-, species-, and subfamily levels, but higher-level syngnathid relationships remain unclear, and recent molecular results are at odds with morphological hypotheses on the evolution of the group. My presentation will review these recent controversies, and discuss how advances in next-generation sequencing and microarray technologies are providing powerful new tools with which to investigate the evolutionary origins of syngnathid fishes and to better understand the functional innovations that have characterized their evolution.

# The conservation genetics of the European long-snouted seahorse *Hippocampus guttulatus*

Lucy Woodall, Paul Shaw, Heather Koldewey

*Project Seahorse/Royal Holloway, University of London, UK, University of Stirling, UK*

This is the first population genetics study on the European long-snouted seahorse *Hippocampus guttulatus*. Patterns of genetic differentiation were determined utilising mitochondrial DNA sequences (control region and cytochrome *b* gene) and microsatellite markers from across the species' geographic range (northeastern Atlantic Ocean, Mediterranean Sea and Black Sea). Analyses revealed significant genetic structuring, which is explored in the context of both contemporary environmental factors and historical processes. The conservation and management of *H. guttulatus* require both regional and international approaches due to high local population density and their large geographic range. The conservation status and current levels of protection for *H. guttulatus* are discussed, and the genetic population structuring observed in this study is used to recommend future conservation and management strategies.

## Abstracts – Poster

Abstracts are listed alphabetically by **presenting author**

# **Molecular characterisation of male pregnancy in the seahorse *Hippocampus abdominalis***

**Marie E. A. Gauthier<sup>1</sup>, Kai N Stölting<sup>2</sup>, Anthony B. Wilson<sup>1</sup>**

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Understanding the evolution of morphological novelty and diversity is a major challenge in evolutionary biology. Seahorses and pipefish (Syngnathidae) possess a unique mode of reproduction in which males incubate developing embryos in specialised brooding structures that display a remarkable range in complexity, from a simple ventral attachment of eggs to a fully enclosed pouch. Therefore, this teleost family presents a useful model for studying the evolution of complex traits. We have designed a custom-made oligonucleotide microarray, derived from 454-based transcriptome sequencing, in order to quantify differences in gene expression associated with pregnant and non-pregnant tissues of the seahorse *Hippocampus abdominalis*. Our probes show highly repeatable hybridisation intensity and our results suggest that male pregnancy is under the regulation of both conserved and taxonomically-restricted genes. A cross-species microarray approach offers an opportunity to investigate the genetic basis of reproductive complexity within syngnathids.

## Male pipefish cannot smell out the females

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In the Baltic Sea eutrophication has become a severe problem with turbid waters as one consequence. Turbidity makes visual cues less effective and thus weakens sexual selection in species using vision to discriminate between partners. It also affects mate encounter rates in species using vision to find each other. A lower mate encounter rate means a weakening of sexual selection and that individuals mate more randomly. One way to compensate for lower visibility would be to use olfactory cues instead. It has previously been shown that males of brood-nosed pipefish *Syngnathus typhle* mate more randomly when water becomes turbid. In this study *S. typhle* from the Baltic Sea population was used to investigate whether males used scent in their search for mates. In an experiment males, which are the searching sex, were provided with a choice between a female and another male. When the focal males had access to visual cues they did choose the female, in contrast to when they had access to scent only, when they made no choice between sexes. Thus vision, but not smell, was used to locate a mate in this species.

## Estimates of population size and site fidelity for syngnathid fishes in Tampa Bay, Florida, USA

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University of Tampa<sup>1</sup>, Texas A & M University<sup>2</sup>, Environ Corporation<sup>3</sup>

We investigated seasonal population size estimates and movement patterns of 5 species of syngnathid fishes in a Tampa Bay, Florida (USA) estuarine system. The main purposes of this work were to identify microhabitats in which these fish are most abundant and to obtain accurate population estimates using population estimation techniques, to identify reasonable conservation targets to better manage their populations on a landscape level. Movement patterns between three sites within the larger site located in a small bay off the South Tampa peninsula were investigated. On each census date, fish were collected by pushnet from a 150m<sup>2</sup> area of seagrass from each of the three sites. Each animal was marked with latex dye under the skin, photographed for body size and reproductive condition estimation, and returned the same day. Recapture rates increased dramatically over our previous study (reflecting a decreased recapture interval), and of the pipefish recaptured, 27% moved between sites, indicating that open population techniques are the most appropriate method of population estimation. No gender differences were observed in movement patterns. For *Hippocampus zosterae*, low recapture rates were observed, with males apparently moving more than females, and data will be presented from a follow-up study to address this issue. With the restriction of *H. zosterae* to the Gulf of Mexico region, accurate population estimates and a thorough understanding of their patchy distribution is critical to management of their population in the wake of the oil spill in April 2010 off of the coast of Louisiana, USA.

## Optimizing *Artemia* enrichment and feeding protocol for rearing juvenile long snout seahorse, *Hippocampus guttulatus*

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The aim of this study was to determine suitable *Artemia* enrichment and feeding protocols for the rearing of juvenile long snout seahorse, *Hippocampus guttulatus* from birth. The first phase of the project, examined the effect of two different *Artemia* enrichments treatments (DHA-Selco®, and *Chlorella* sp.) on the growth performance and survival of early-stage juvenile *H. guttulatus*. DHA-Selco® enrichment of *Artemia* resulted in a null survival at 10 DPP whereas the use of *Artemia* enriched with *Chlorella* sp. resulted in 20% survival at the end of 60 DAH. The second phase of the study (60 to 120 DAH) reported the progressive feed transition of juvenile seahorse from *Artemia* to live shrimp (*Palaemonetes varians*) and from live to frozen shrimp and its effect on growth performance and survival of the juveniles. Overall, the mean standard length of juvenile *H. guttulatus* increased from  $1.4 \pm 0.05$  cm at release from male's pouch to  $12.4 \pm 1.2$  cm at 120 DAH. Mean juvenile wet weight increased from  $4.2 \pm 0.2$  mg to  $4133 \pm 1230$  mg in that same period. Length, wet weight, daily weight gain and thermal-unit growth coefficient were used to characterise juvenile *H. guttulatus* growth performance, which was found to be similar to that of published accounts for other juvenile seahorse species at the same life stage. This study is the first record of successful rearing of *H. guttulatus* juveniles in captivity, and offers a promising starting point to improve the rearing techniques to successfully breed this candidate aquaculture species.

## Using enriched shrimp as a diet for the long snout seahorse (*Hippocampus guttulatus*)

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This investigation examined the effect of enriched shrimp diets on the fitness condition of young adult long snout seahorse (*Hippocampus guttulatus*). Three shrimp diets 1) natural shrimp (control diet), 2) 10-day enriched shrimp and 3) enriched shrimp (with ingested artificial diet) were fed to seahorses during a 12 week period. At the end of the experiment, significant differences on the final wet weight were found between seahorses fed the three different treatments ( $F_{3,54}=5.56$ ,  $P<0.009$ ). Significant differences were found between seahorses fed enriched shrimp with ingested artificial diet (co-feed) and those fed either natural shrimp or 10-day enriched shrimp ( $P<0.001$ ), but no differences were found between natural shrimp and 10-day enriched shrimp treatments ( $P>0.05$ ). Seahorses fed enriched shrimp with ingested artificial diet had a significantly higher ( $F_{3,54}=3.52$ ,  $P<0.05$ ) weight gain increase (67.3%) than seahorses fed natural shrimp and 10-day enriched shrimp (50% and 46.6%, respectively). Thermal Growth Rate (TGC) and Food Conversion Rate (FCR) showed the same pattern of statistical significance, as significant differences were found among treatments (TGC;  $F_{3,54}=3.59$   $P<0.05$ , FCR;  $F_{3,54}=7.14$ ,  $P<0.003$ ). This study showed that the combined use of a natural diet (shrimp) co-fed with an artificial diet, not only provides good growth rates, but it is also a practical co-feeding of two diets in just one item. Thus, it can be considered as a step ahead in seahorse nutrition.



## **Effect of broodstock nutrition on the long snout seahorse (*Hippocampus guttulatus*) reproductive rate and brood quality**

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This study examined the effect of long snout seahorse (*Hippocampus guttulatus*) broodstock nutrition on the reproductive rate and brood quality. Three natural diets were fed to seahorses during a 12 week period: natural shrimp (control diet) (Diet A); 10-day enriched shrimp (Diet B) and shrimp co-fed with an ingested artificial dry diet (Diet C). At the end of the experiment, adults fed Diet C grew significantly more ( $P < 0.01$ ) than those fed Diet A and Diet B. Adult seahorses fed Diet C generated more broods (9), more fries per brood ( $299 \pm 87$ ) and significantly bigger fries ( $18.1 \pm 0.1$  mm standard length) than adults fed Diets A and B. Multivariate statistical analyses were used to discriminate juvenile origin based on three morphological traits (head, trunk and tail length). This analysis indicated significant differences in the morphometry of juveniles hatched from parents fed the three different dietary treatments (Wilk's  $\lambda = 0.2$ ,  $F(6,460) = 47.41$ ,  $P < 0.0001$ ), and all three measurements were selected for sample discrimination. This study showed that adult seahorses co-fed with a combination of a natural and artificial diet, generated better broods and more fitted descendents, fact that is extremely relevant within the seahorse nutrition and production.

# Does the paternal uptake of embryonic nutrients lead the sexual difference in the trophic position? Application of stable isotope analysis

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Recent study showed that males of the broad-nosed pipefish, *Syngnathus typhle*, absorb nutrients derived from brooded embryos through their brood pouch. If such a paternal uptake occurs generally in wild and brooded embryos constitute a significant contribution to the nutritional source for brooding males, males would occupy a higher trophic position than females during the breeding season. In the present study, we estimated the trophic position of a pipefish, *S. schlegeli* collected from some populations along the coast of Japan, by examining nitrogen stable isotope ratios ( $\delta^{15}\text{N}$ ) of muscle, liver and mature eggs in the ovary. We predicted that the  $\delta^{15}\text{N}$  values are higher in males than females if the paternal uptake of embryonic nutrients forms a significant proportion of total nutritional intake for brooding males. We also examined whether there is a geographical variation in the sexual difference in the trophic position.

## **Spatial variation in stable isotopes of *Hippocampus guttulatus* in the Galician coast (NW Spain)**

**Sonia Valladares, M. Planas**

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Trophic ecology studies in fishes usually involve the undesirable death of the animal, or field direct observations. The use of stable isotope analysis (SIA) has emerged as an important tool in the study trophic ecology. Due to the conservative status of seahorses (endangered species included in the IUCN Red List Category and Criteria), it is necessary to use a nonlethal sampling technique for the study of their trophic ecology. Fin tissue sampling has become a useful nonlethal practice recently used in SIA analyses of fish instead of lethally sampling procedures. We analysed stable isotopes values of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) in dorsal fin tissues of long-snouted seahorses *H. guttulatus* from four different sites (S1: Aldán; S2: Bueu; S3: Punta Cabalo and S4: Toralla) in the Galician coast (NW Spain). A total of 18 seahorses were sampled from February to March 2010. No sex differences were found neither for  $\delta^{15}\text{N}$  ( $p=0.35$ ) and  $\delta^{13}\text{C}$  ( $p=0.56$ ) values. Comparisons between sites did not show significant differences for  $\delta^{13}\text{C}$  (S1:  $-15.58 \pm 0.17$ ; S2:  $-16.03 \pm 0.40$ ; S3:  $-15.57 \pm 0.19$  and S4:  $-15.94 \pm 0.36$ ). On the contrary,  $\delta^{15}\text{N}$  values were significantly different between sites ( $F=3.72$ ,  $p=0.04$ ). Post-hoc comparisons showed that nitrogen signatures in S4 ( $12.07 \pm 0.23$ ) were higher than in S1 ( $11.61 \pm 0.19$ ) and S2 ( $11.44 \pm 0.30$ ). Differences in  $\delta^{15}\text{N}$  suggest either that the seahorses from S4 feed on prey with higher trophic level or that isotopic signatures of the prey are lower in S1 and S2.

## Description of *Hippocampus guttulatus* populations in Punta Cabalo (Ría de Arousa, NW Spain)

**Sonia Valladares**, P. Quintas, A. Blanco, A. Chamorro, T. Hermelo, M. Planas

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The long-snouted seahorse *Hippocampus guttulatus* Cuvier 1829 and *H. hippocampus* (L., 1758) are the only two seahorse species of seahorses occurring along the coast of Spain where the present study was carried out. Some previous studies were focussed on wild populations of *H. guttulatus* in Portugal but the available information on wild populations in Spanish waters is lacking. In the present study the distribution and composition of *H. guttulatus* populations in Punta Cabalo (Ria de Arousa, NW Spain) were described. A total of 22 sampling surveys were conducted in the study area between June 2009 and August 2010. Underwater visual census surveys were conducted by SCUBA diving covering an area of 3000 m<sup>2</sup> in shallow waters (<10 meters in depth). Sex, maturity sexual, GPS position, depth, habitat preference, weight and length data were recorded for all seahorses. VIFE tags were used to identify each seahorse. Cirrus and dorsal fin tissues were collected for further genetic and stable isotope analyses, respectively. A total of 21 seahorses (11 males, 7 females and 3 juveniles) were recorded. Seahorses were mainly observed in sand bottoms attached to different macroalgae (*Sargassum muticum*, *Dictyota* sp. and *Chondrus crispus*), with weights ranging from 8,06 - 23,06g in males, 11,95 - 13,78g in females and 3 - 5,54g in juveniles. During the period of study, five of those seahorses were recaptured for several times. Recaptured seahorses were located at similar GPS positions.

## **First observations of Siamese-twins in newborn seahorses (*Hippocampus guttulatus* Cuvier, 1829)**

A Blanco, P. Quintas, **Sonia Valladares**, M. Planas

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Three Siamese-twins (ST1, ST2 and ST3) were released by three pregnant seahorses (N81, N104 and N105, respectively) (*Hippocampus guttulatus* Cuvier, 1829), hand-caught collected in Galicia (NW Spain). Although the occurrence of Siamese twins among fish, both in the wild and under culture conditions, was recorded in previous studies, the findings we presented here are the first record of conjoined-twins in seahorses. All three Siamese-twins (summer-autumn 2010) were found to be in the post yolk sac-stage with different developmental stage. Male N81 mated in the laboratory some days after collection. Males N104 and N105 were already pregnant when collected in the wild. ST1 twins, each twin had their own pectoral and dorsal fin and a unique well developed tail. ST2 twins had a separated well developed head, a fused trunk from the jaw and a completely deformed and twisted tail. ST3 twins showed an underdeveloped head which appeared to be fused to the abdominal region of the other twin. Total lengths in twins were smaller, particularly for ST3, than in normally developed newborns. As for most abnormalities in early developmental stages of fish, the occurrence of twinning can be due to a wide range of causes, such as egg over-ripening or pollutants. Fish are sensitive to possible teratogenic effects causing mitotic abnormalities on embryos or specific locus mutations during the oogenesis and spermatogenesis. Polyembryonism in fish occurs also in nature and our findings demonstrate for the first time the occurrence of that feature in wild seahorses.

## Biology of *Syngnathus typhle* in Greece

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Syngnathid fishes have evolved particularly pronounced adaptations for male parental care. During copulation, a female transfers eggs to a male's ventral surface where they are fertilized and brooded. In the present study population structure and growth of *Syngnathus typhle* was studied in the littoral zone of the North Ionian Sea, Greece. From July 2008 to March 2010 were collected 887 specimens. The sample composed of 329 (37.09%) male, 348 (39.23%) female and 209 (23.56%) juvenile and unsexed specimens; the sex ratio was M : F = 1:1. The population composed of specimens from 2.4 cm (juvenile) to 24.3 cm (female). The slope of the length-weight relationship was 3.21 (95% c.i: 3.14- 3.29) indicating a positive allometric growth of the population. No difference among sexes was found. The spawning season lasts from May to August. The highest values of the gonadosomatic index were recorded in April while the peak of the brooding period was in July and August. The hepatosomatic index showed the highest values in March and April for females and males respectively and the lowest in November for both sexes. Finally the genetical structure of the species was studied. The present study is the first record on the biology of *Syngnathus typhle* in Greece.

## **Workshop: IUCN Red Listing for Syngnathids**

**Chair: Amanda Vincent**

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The conservation status of most syngnathids has never been evaluated. This optional two hour workshop will provide training and support for participants to undertake IUCN Red List assessments on species' conservation status ([www.redlist.org](http://www.redlist.org)). During the first hour, Dr. Vincent will introduce and explain categories and criteria for Red Listing, with particular reference to marine fishes. In the second hour, participants will discuss methodology, sample assessments and effectiveness for syngnathids. Participants should leave with a general comfort with Red Listing.





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