



The effect of temperature on mate preferences and female–female interactions in *Syngnathus abaster*

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(Received 12 October 2006; initial acceptance 5 December 2006;

final acceptance 8 March 2007; published online 24 September 2007; MS. number: 9140R)

Despite much effort to ascertain the consequences of temperature variation for a wide range of animal performance traits, the effect of temperature on interactions among organisms is still poorly understood. The present work tests for a direct influence of water temperature on sexual recognition, mate preferences and female–female interactions in the pipefish, *Syngnathus abaster*. Three experiments were conducted by monitoring time spent in the vicinity of conspecifics at three water temperatures intended to reflect sea water temperatures before the onset of reproduction (15°C) and during the early (18°C) and late breeding season (24°C). Four major results emerged: (1) *S. abaster* can visually discriminate potential mates from fish of the same sex; (2) males and females responded differently with the former diverting their attention towards the opposite sex at intermediate (18°C) and high temperatures (24°C), while the latter only showed a significant interest in potential mates at 24°C, devoting an equal amount of interest towards both males and females at 18°C; (3) at breeding season water temperatures, both sexes discriminated against smaller partners, preferring larger ones; (4) different-sized females adopted distinct temperature-modulated behavioural responses, possibly because large dominant females, which engage in competition at 18°C, constrained the reproduction of smaller ones, which seem to compete only at 24°C. These results highlight the importance of temperature as an effective agent in the modulation of *S. abaster* reproductive behaviour. Considerations on the ecological significance of the observed behavioural patterns are also discussed.

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Keywords: female–female competition; mate choice; pipefish; reproduction; sexual selection; Syngnathidae; *Syngnathus abaster*; water temperature

The decision-making processes involved in mating behaviour can have profound implications on the dynamics of sexual selection, the mechanism of evolution and speciation (Andersson 1994). Although traditional theoretical models have generally ignored the consequences of

individual variation in mate choice, it is known that there are both genetic and environmental conditions capable of inducing polymorphism in mating decisions (Jennions & Petrie 1997). In fact, mate choice behaviour is a contextual phenomenon moulded by a variety of factors, such as the genotype of the choosing individual and/or that of the potential mate or mates (Puurtinen et al. 2005), time and energy costs of sampling (Milinski & Bakker 1992), social environment (Galef & White 2000) and predation risk (Berglund 1993). Studies in a variety of fish species have shown that environmental abiotic features, such as oxygen concentrations (e.g. *Pomatoschistus microps*: Reynolds & Jones 1999), light (e.g. *Gasterosteus aculeatus*: Rick et al. 2006), water turbidity (e.g. *Pomatoschistus minutus*: Jarvenpaa & Lindstrom 2004) and temperature, can be effective agents in the modulation of mating behaviour. In

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sand gobies, *P. minutus*, a trend towards a higher frequency of female–female interactions was observed in the cold water treatment, suggesting that females were more liable to compete when the water was cold. When the water was warm, by contrast, females interacted less and appeared to concentrate on carefully choosing a partner (Kvarnemo 1996).

Despite much effort to determine the functional consequences of temperature variation for a wide range of animal performance traits, there is still little understanding of the influence of thermal variation on behavioural interactions among organisms (Wilson 2005), a topic of increasing importance in light of the implications of increasing sea water temperatures as an expected consequence of global warming (Walther et al. 2002). The present work tries to determine the influence of water temperature in the ability of *Syngnathus abaster* to recognize and select mating partners. Additionally, an alteration in female–female interaction patterns concurrent with increasing water temperatures was also investigated. Experiments were conducted using three distinct temperatures, intended to reflect sea water temperatures before the onset of reproduction and during the early and late breeding season of a Southern European *S. abaster* population. Furthermore, considerations on the ecological significance of the observed behavioural responses are presented.

METHODS

Syngnathus abaster is a euryhaline species with a restricted distribution that includes the Mediterranean, the Black Sea, and the Atlantic coast of South West Europe up to southern Biscay (Dawson 1986). This black-striped pipefish occurs either in coastal areas or in brackish and fresh waters (Cakic et al. 2002), and can be found mainly among sand, mud or eelgrass meadows, at depths between 0.5 and 5 m, within a temperature range of 8–24°C. Males have a brood pouch located ventrally on the tail (Urophori) which consists of two skin folds that contact medially with their free edges. Sex roles seem to be reversed, as females are larger and apparently more competitive than males, at least under even sex ratio conditions (Silva et al. 2006).

Fish were collected with a hand net, in a salt pond reservoir, at the Ria de Aveiro estuarine lagoon (40°45'N, 8°40'W), in Portugal, and transported to the laboratory, where they were maintained in 250-litre aquaria illuminated by natural light supplemented with 18-W fluorescent lamps. Tank substrata consisted mainly of sand and plastic seagrass laid to mimic the original habitat where the fish were caught. The continuously running sea water was physically and biologically filtered and aeration was performed outside the fish tanks to prevent the 'gas bubble disease', common in pipefish (Monteiro et al. 2002). Fish were fed daily with fresh *Artemia franciscana* nauplii. Sexually mature males and females were kept in separate tanks.

Three aquarium experiments were conducted using three distinct water temperatures intended to mimic the periods immediately before the onset of reproduction (15°C), the beginning of the breeding period (18°C) and

the final stage near the end of the reproductive season (24°C). These values were obtained and validated in the field. At 15°C no pregnant males were found, with eggs appearing in the marsupium only when temperatures reached ≈17°C. Reproduction ceases shortly after temperature reaches its highest values (≈25°C). In the stock tanks the fish were kept at 18°C, a temperature at which they breed continuously. Before the experiments at 15°C or 24°C, the fish were first placed in new aquaria at 18°C, after which the temperature was gradually increased or decreased during a period of 10–15 days. After the tanks reached the desired temperature, the fish were left to acclimate for a period of 1 month before the experiments were conducted. It is important to note that at 24°C the fish continue to breed regularly, while at 15°C reproduction ceases.

Size cut-offs for 'large' and 'small' individuals were defined as ½ standard deviation (SD) below and above the mean size (total length) for each sex (♀: mean = 9.4 cm, SD = 1.38 cm; ♂: mean = 8.5 cm, SD = 1.26 cm). Large males and females were longer than 9.1 and 10.1 cm, respectively. Small males and females were shorter than 7.9 and 8.7 cm, respectively. Since the smallest observed pregnant male was 5.2 cm, it seems likely that the smallest individual used (6.5 cm) was also mature.

Because of the large number of individuals needed to complete all three experiments, some of the focal fish were reused in posterior experiments as stimulus fish. Since stimulus fish were kept inside two smaller aquaria (ruling out the possibility of chemical communication) and were also unable to see the focal fish, it can be assumed that this procedure could not have altered the observed response patterns.

Experiment 1

The first experiment consisted on a tendency evaluation of different-sized males and females to approach consensual and nonconsensual individuals at three preselected water temperatures. Experimental set-up is shown in Fig. 1a: a one-way mirror between aquarium 1 and the other two smaller aquaria (2 and 3) allowed the focal fish in aquarium 1 to see the stimuli fish without being seen, ruling out the possibility of a response based on chemical communication. An opaque wall located between the two smaller aquaria blocked visual contact between the two stimuli individuals. Two distinct areas were considered in aquarium 1, with area A located closer to the one-way mirror (see Fig. 1a) than area B ('neutral' area). The time spent in area A, directly in front of one of the two smaller aquaria (aquarium 2 or 3, randomly containing a male or a female; see Fig. 1) was monitored during 30 min, after an initial acclimatization period (≈45 min). Tests started only when the observer, located at 50 cm from aquarium 1, was confident that the focal fish showed no stress signs and had had the opportunity to see the stimuli fish, which were matched for size. Stimuli fish were randomly allocated to aquaria 2 and 3 to rule out possible effects of other variables on focal fish behaviour. The 'relative interest' (time spent in Area A in front of aquarium 2 or 3/1800 s) was measured for 20 males and 20 females (10 large and 10 small individuals)

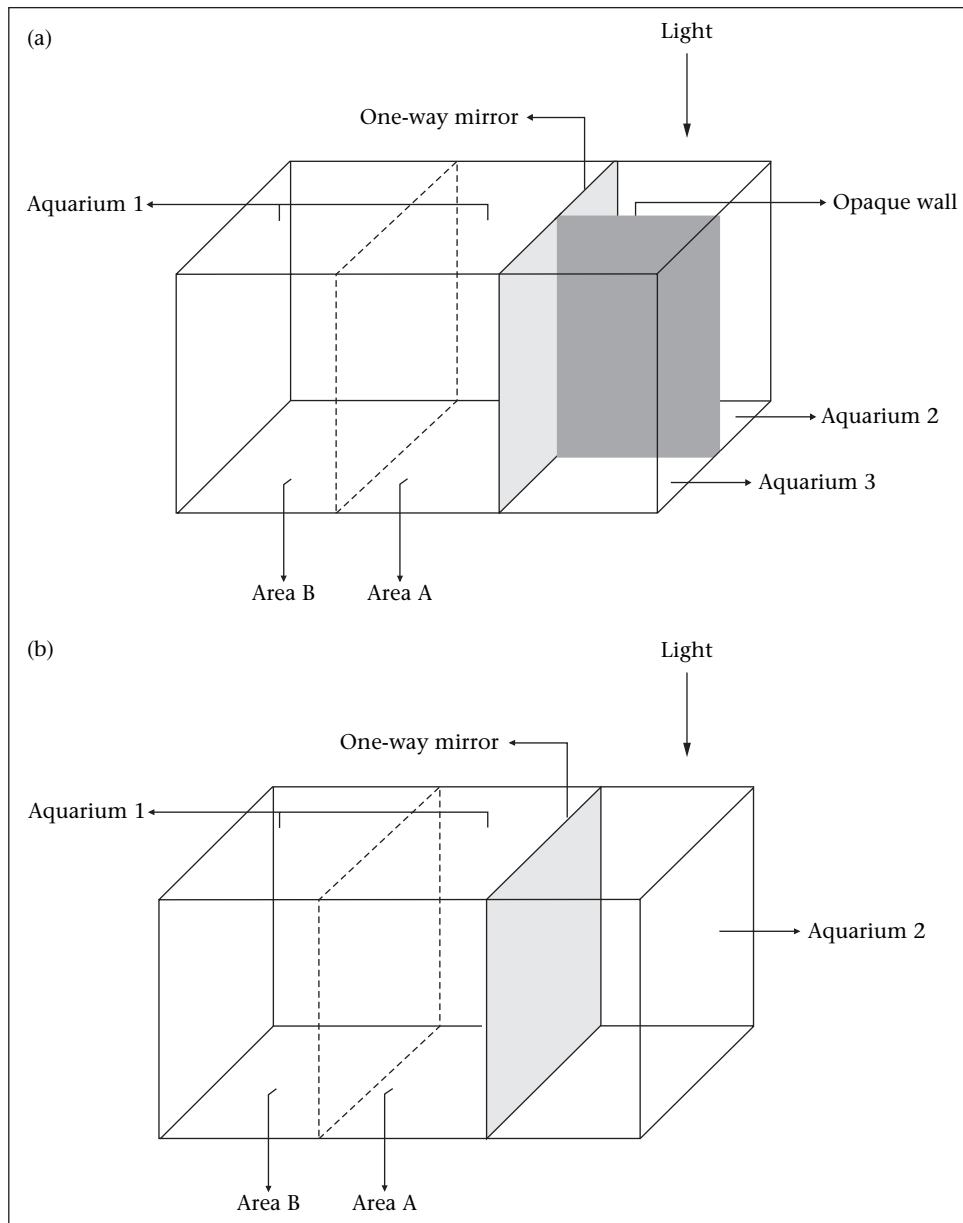


Figure 1. (a) Experimental set-up used in experiments 1 and 2; (b) experimental set-up used in experiment 3.

for each temperature. Different focal individuals were used in each trial to avoid data dependency. Each set of stimulus fish (one male and one female) was presented to a pair of focal fish alternatives (once for a male and once for a female), after which they were not further reused (see also Methods: statistical analysis).

Experiment 2

Mate preferences for body size were analysed through a protocol similar to that of experiment 1. Males and females were simultaneously presented with two potential mates of different sizes (one large and one small), and 'relative interest' displayed by focal fish was recorded for each temperature. Twenty focal males and 20 focal females (10 large and 10 small individuals) were used. As in experiment 1 the stimuli fish were randomly allocated

to aquaria 2 and 3. Different focal individuals were used in each trial to avoid data dependency. Each set of stimulus fish (one large and one small) was presented to a pair of focal fish alternatives (once for a large and once for a small individual), after which they were not further reused (see also Methods: statistical analysis).

Experiment 3

From the analysis of data collected in experiments 1 and 2, experiment 3 was planned to study the 'relative interest' of focal females on other females with different body sizes (small and large). Experimental set-up was composed of two aquaria (Fig. 1a) with one focal female (aquarium 1) separated by a one-way mirror from a stimulus female (aquarium 2). Aquarium 1 was also divided in two areas, one adjacent to the mirror (area A) and one 'neutral

zone' (area B). Large and small focal females (20 large females and 20 small females, for each temperature) were presented with stimuli females of two size classes (large and small) and 'relative interest' was measured (suggesting female–female competition). In this experiment, each focal female was exposed only to a single stimulus female, either a large or a small one. Different focal females were used in each trial to avoid data dependency. Each stimulus female was presented to a pair of focal fish alternatives (once for a large and once for a small individual), after which they were not further reused (see also Methods: statistical analysis).

Statistical Analysis

All statistical analyses were performed using Statistica 7.0 (Statsoft Tulsa, OK, U.S.A.). Data from the three experiments were tested for deviance from normality and for the homogeneity of variance. Assumptions were met in experiment 2 but no homogeneity of variance was found in experiments 1 and 3 (Levene test; $P < 0.05$). Since, according to Lindman (1974), the F statistic is quite robust against this violation when there is not a significant correlation between means and variances across the cells of the design (Spearman correlation; experiment 1: $R = 0.3747$, $P > 0.05$; experiment 2: $R = 0.0140$, $P > 0.05$), parametric statistics were used throughout the entire study. Experiment 1: orthogonal analysis of variance (ANOVA) with four factors; temperature (three levels = 15°C, 18°C and 24°C), sex of focal fish (two levels = male and female), size of focal fish (two levels = large and small) and sex of stimulus fish (two levels = male and female). Experiment 2: orthogonal ANOVA with four factors; temperature (three levels = 15°C, 18°C and 24°C), sex of focal fish (two levels = male and female), size of focal fish (two levels = large and small) and size of stimulus fish (two levels = large and small). Experiment 3: orthogonal ANOVA with three factors: temperature (three levels = 15°C, 18°C and 24°C), size of focal female (two levels = large and small) and size of stimulus female (two levels = large and small). Post hoc comparisons were conducted using Newman–Keuls test. All probabilities are two-tailed and a significance level of 0.05 was used.

Ethical Note

All the focal and stimuli fish were returned to stock tanks after the experiments and no signs of disturbance or stress could be detected. No fish died or became sick and no manipulations were used in the study apart from that needed to transfer the fish from one tank to another with a small hand net. The study was conducted in agreement with the pertinent European and Portuguese legislation on animal welfare.

RESULTS

Experiment 1

A significant interaction was found between temperature, sex of the focal fish and sex of the stimulus fish (see

Table 1. Analysis of variance results on the 'relative interest' of males and females of different size classes towards conspecifics and nonconspecifics, at three different temperatures (15°C, 18°C and 24°C; experiment 1)

Source	df	Mean square	F	P
Temperature (Temp)	2	0.48	5.33	0.006
Sex of the focal fish (Sex_F)	1	0.16	1.77	0.187
Size of the focal fish (Size_F)	1	0.01	0.06	0.811
Sex of the stimulus fish (Sex_St)	1	0.44	4.84	0.030
Temp*Sex_F	2	0.19	2.12	0.125
Temp*Size_F	2	0.04	0.48	0.621
Sex_F*Size_F	1	0.12	1.39	0.242
Temp*Sex_St	2	0.63	7.00	0.001
Sex_F*Sex_St	1	3.94	43.75	0.000
Size_F*Sex_St	1	0.02	0.20	0.660
Temp*Sex_F*Size_F	2	0.20	2.20	0.116
Temp*Sex_F*Sex_St	2	0.63	6.70	0.001
Temp*Size_F*Sex_St	2	0.01	0.07	0.928
Sex_F*Size_F*Sex_St	1	0.01	0.16	0.692
Temp*Sex_F*Size_F*Sex_St	2	0.16	1.82	0.167
Error	96	0.09		

Table 1). At higher temperatures (24°C), both males and females spent more time in front of the opposite sex (Fig. 2c, d), while at 15°C no significant differences were observed (Fig. 2a, d). At 18°C, a distinctive pattern was observed, with males spending more time in the vicinity of the opposite sex and females spending an approximately equal amount of time in the vicinity of both conspecifics and nonconspecifics (Fig. 2b, d). In males, 'relative interest' in females increased as soon as water temperature was raised to 18°C (temperature that mimics the onset of the breeding season), while in females a clear interest on males was visible only at 24°C.

Since, at least at higher temperatures, both males and females seemed to effectively discriminate among sexes (Fig. 2), experiments 2 and 3 were conducted.

Experiment 2

A significant interaction was found between temperature and size of the stimulus fish (see Table 2). At lower temperatures (15°C), no significant differences were found between the 'relative interest' aroused by either size class. However, as temperatures increased, larger stimuli fish obtained more attention from focal individuals. No differences between temperatures were found for small fish (see Fig. 3a, b). Furthermore, independently of temperature, males spent more time near the stimuli fish than females (see Table 2).

Experiment 3

Three significant interactions were found (see Table 3):

(1) Temperature and size of the focal females: at 15°C, no significant differences were found between different-sized focal females' responsiveness to stimuli females. On the other hand, at 18°C, large females spent significantly

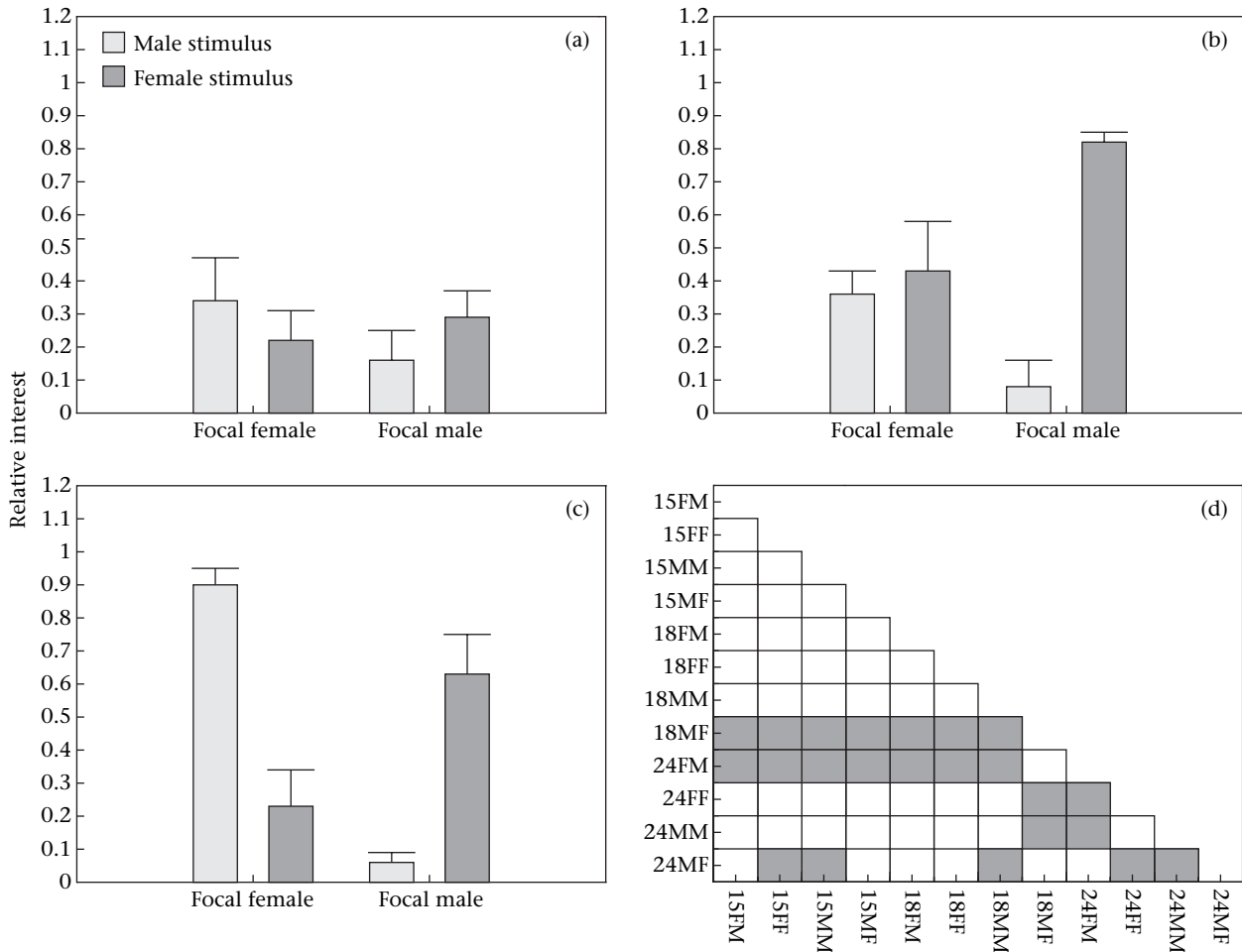


Figure 2. ‘Relative interest’ of males and females of different size classes towards consensuals and nonconsensuals, at three different temperatures (a, 15°C; b, 18°C; c, 24°C) and Newman–Keuls test results (d), with grey squares representing significant differences. The numbers 15, 18, 24 represent the tested temperatures and the two initials (with F for females and M for males) represent the focal and the stimulus fish, respectively. Error bars represent standard deviations.

Table 2. Analysis of variance results on the ‘relative interest’ of males and females of different size classes towards large and small potential mates, at three different temperatures (15°C, 18°C and 24°C; experiment 2)

Source	df	Mean square	F	P
Temperature (Temp)	2	0.52	5.98	0.004
Sex of the focal fish (Sex_F)	1	0.54	6.16	0.015
Size of the focal fish (Size_F)	1	0.05	0.58	0.450
Size of the stimulus fish (Sex_St)	1	2.91	33.27	<0.001
Temp*Sex_F	2	0.41	0.47	0.624
Temp*Size_F	2	0.20	2.34	0.102
Sex_F*Size_F	1	0.60	0.68	0.411
Temp*Sex_St	2	0.35	3.99	0.022
Sex_F*Sex_St	1	0.16	1.88	0.174
Size_F*Sex_St	1	0.11	1.23	0.270
Temp*Sex_F*Size_F	2	0.06	0.72	0.489
Temp*Sex_F*Sex_St	2	0.07	0.83	0.441
Temp*Size_F*Sex_St	2	0.08	0.87	0.420
Sex_F*Size_F*Sex_St	1	0.14	1.63	0.205
Temp*Sex_F*Size_F*Sex_St	2	0.24	2.77	0.067
Error	96	0.09		

more time in area A than small females. At high temperatures (24°C) an inverted pattern was observed, with small females spending significantly more time in area A than large females (Fig. 4a, b).

(2) Temperature and size of the stimuli females: at low temperatures (15°C) large females attracted significantly more attention than small females. No significant differences were found either at 18°C or at 24°C (Fig. 4c, d).

(3) Size of the focal females and size of the stimulus females: large females concentrated their attention on females of equivalent size, while small females show no distinct preference (Fig. 4e, f).

DISCUSSION

Mating discrimination is the ability of individuals of one sex to identify, assess and selectively mate with conspecific members of the opposite sex (Andersson 1994; Pfennig 1998). Attempts to quantify mating preferences and/or intrasexual interactions often rely on restrained access experiments where individuals are presented with different stimuli (Wagner 1998). However, objective interpretations of such experiments cannot be obtained

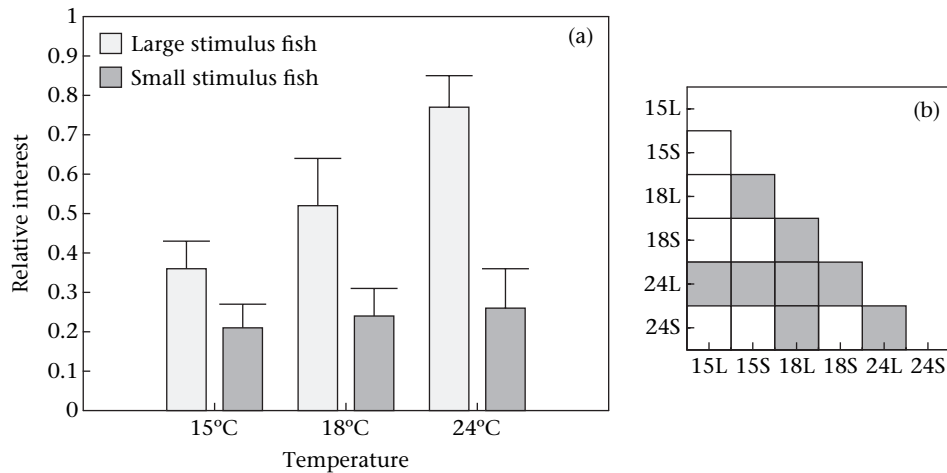


Figure 3. 'Relative interest' towards stimulus fish of different sizes (a) at three different temperatures (15°C, 18°C and 24°C) and Newman-Keuls test results, with grey squares representing significant differences (b). The numbers 15, 18, 24 represent the tested temperatures and the two initials (with F for females and M for males) represent the focal and the stimulus fish, respectively. Error bars represent standard deviations.

without confirmation of mate discrimination. Furthermore, the outcome of such experiments may be modified by environmental factors, such as temperature (e.g. Kvarnemo 1996).

Besides showing that *S. abaster* can discriminate potential mates from fish of the same sex using only visual information (observed fish were kept inside a transparent sealed tank), the results of experiment 1 also provide evidence on how a physical factor, water temperature, can directly and differentially modulate the behavioural responses of *S. abaster* males and females when exposed to conspecifics (see Fig. 2).

Even though some exceptions can occur (see Kidd et al. 2006), physical proximity has previously been validated as a reliable indicator of mate choice (Forsgren 1992; Kodric-Brown 1993; Berglund 1994; Gonçalves & Oliveira 2003; Wong 2004). Curiously, *S. abaster* males and females exhibited different response patterns, especially at water temperatures that mimic the onset of the breeding season. While male interest in females was expressed at 18°C, female interest at this temperature was distributed among both males and females, while at 24°C interest towards

males was significantly greater than that towards conspecifics. One possible hypothesis to explain these results would involve a difference between sexes in the way sexual behaviour and the underlying motivational mechanisms vary with temperature. It is known that syngnathid females ready to mate spend a considerable amount of time interacting with other females (Berglund & Rosenqvist 2003). It is possible that at 18°C the mechanisms favouring approaching males and those involved in female–female interactions compete, so that sexual motivation is partly masked in females. At 24°C, the motivation to approach males would become dominant, with females spending less time with conspecifics. In males, in which sex-role reversed syngnathids often have no need to compete with other males (Berglund & Rosenqvist 2003), the mechanism could be simpler, basically translated into an increased focus on females. This difference between male and female response to temperature makes sense in the environmental conditions where *S. abaster* lives. In fact, this species breeds during summer, with temperatures increasing rapidly to a peak late in the season and dropping visibly in the beginning of autumn, when reproduction no longer takes place (K. Silva, personal observation). In this context, females that still have eggs to spawn by the end of the breeding season would likely gain by minimizing time spent in interactions with other females and concentrating their efforts in courting males. Alternatively, it is possible that a mating hierarchy is enforced, especially during an initial phase of the breeding season, thus explaining the later decrease in interest towards other females.

As in other pipefish species (Berglund & Rosenqvist 2003), body size proved to be an important trait in *S. abaster*. According to the results of experiment 2, at 18°C and 24°C (temperatures at which reproduction occurs) both males and females spent more time near larger potential partners, than what happened at 15°C, where no preferences were observed. Furthermore, as temperature increased, larger individuals tended to obtain more attention from the focal fish, independently of the size and sex

Table 3. Analysis of variance results on the 'relative interest' of females of different size classes towards large and small conspecifics, at three different temperatures (15°C, 18°C and 24°C; experiment 3)

Source	df	Mean square	F	P
Temperature (Temp)	2	0.06	1.12	0.329
Size of the focal female (Size_F)	1	0.52	9.68	0.002
Size of the stimulus female (Size_St)	1	0.39	7.18	0.009
Temp*Size_F	2	1.01	18.69	<0.001
Temp*Size_St	2	0.18	3.29	0.041
Size_F*Size_St	1	0.39	7.25	0.001
Temp*Size_F*Size_St	2	0.00	0.03	0.969
Error	108	0.05		

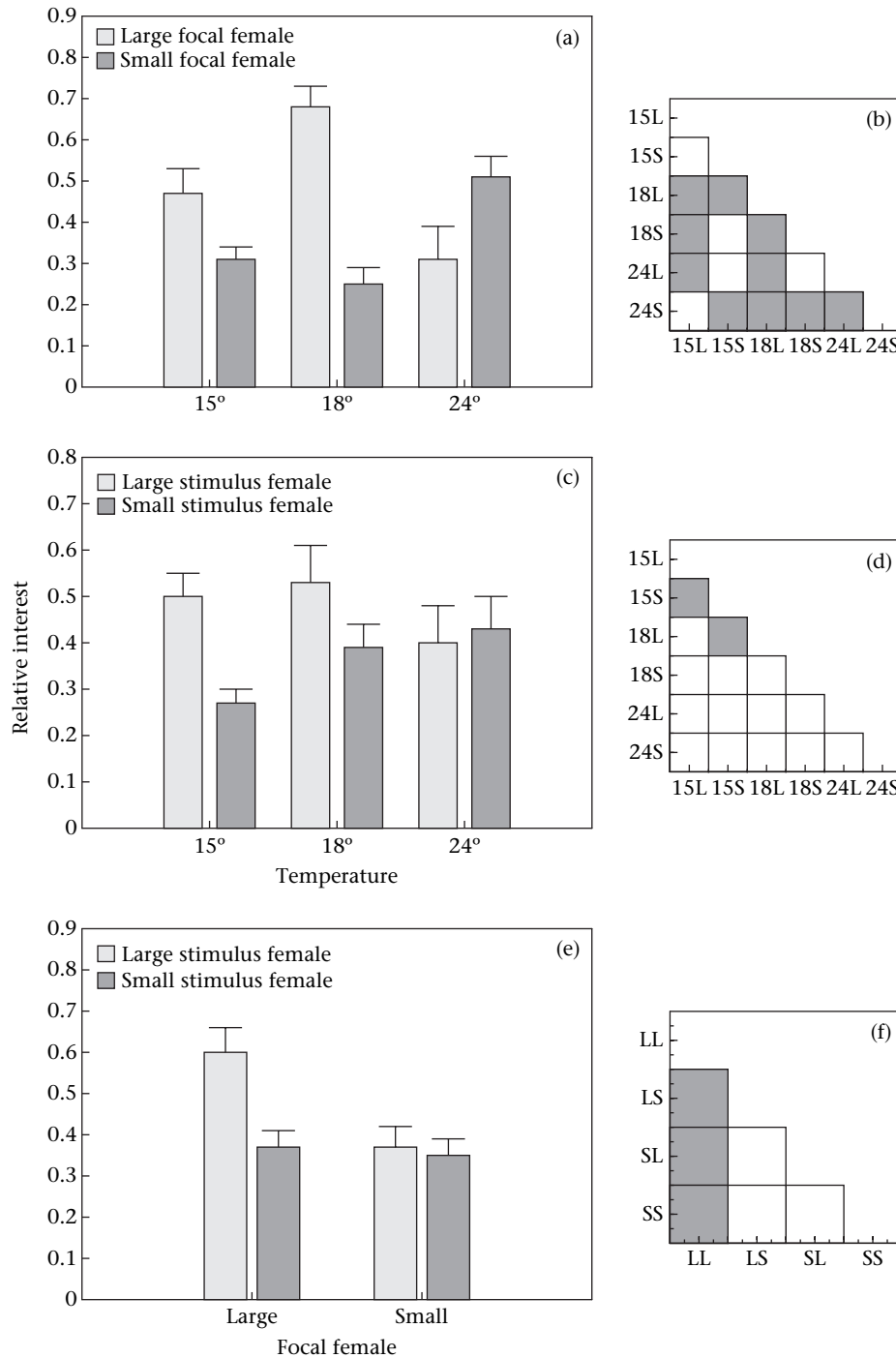


Figure 4. ‘Relative interest’ of females of different size classes towards stimuli females (a), at three different temperatures (15°C, °C and 24°C). ‘Relative interest’ aroused by females of different size classes (c) at three different temperatures (15°C, 18°C and 24°C). ‘Relative interest’ of different size females towards large and small conspecifics (e). (b), (d) and (f) represent Newman–Keuls test results, with grey squares showing significant differences (for a, c and e, respectively). The numbers 15, 18, 24 represent the tested temperatures and the two initials (with F for females and M for males) represent the focal and the stimulus fish, respectively. Error bars represent standard deviations.

of the latter. By discriminating against smaller partners, individuals could benefit in several ways. In *S. typhle*, larger size means higher quality in terms of reproductive return for both males and females, since both sexes accrue direct advantages from mating with larger individuals (Jones et al. 2000; Berglund et al. 2005).

Results of experiment 2 also showed that males spent significantly more time in the vicinity of females than females in the vicinity of males (see Table 2). Intuitively, in a sex-role reversed species, as seems to be the case of *S. abaster* (Silva et al. 2006), one would expect females to be more active than males in approaching the opposite

sex. However, it is important to distinguish between situations in which females have difficulty finding a male ready to receive eggs and those in which the males have their brood pouch empty like in experiment 2. In this case, males may express a courting motivation that would be masked in experimental designs aimed to emphasize female competition. In fact, Silva et al. (2006) showed that *S. abaster* males have an active role in mating, approaching and courting females.

Exhibiting a preference and actually succeeding in pursuing the mate choice can be two very different things. Individual behaviours may be governed by factors beyond the individual's control, such as competition from others (Berglund et al. 2005). In sex-role reversed syngnathids, where males are generally viewed as a limiting factor and no conspicuous agonistic interactions occur, females should assess their attractiveness relative to other females to establish a hypothetical status that reliably translate their intrinsic quality. If this is the case, then the still poorly understood female–female interactions, that vary considerably among syngnathid genera, may play an important role in the competition for access to mates. In *N. ophidion* and *S. typhle*, female's competition for males is mainly indirect, through dominance hierarchies, with larger females dominating smaller ones by sexual signalling, namely a more contrasted coloration in the trunk (an amplifier of a previous quality signal such as body size, which is also correlated with fecundity; Berglund & Rosenqvist 2003). Moreover, as predicted in a model of mutual mate choice as a dynamic game, proposed by Johnstone (1997), which yielded predictions about mating behaviour under the influence of time constraints, choice costs and competition for mates, when all individuals are present from the start of the breeding season, the correlation between the qualities of individuals pairing at a given time declines throughout the season, so that mates are more closely matched among individuals who pair early than among those who pair late. The mean quality of unmated males and females declines over time, because more attractive individuals tend to mate sooner. If this is so, then it is possible that in *S. abaster* the chance for small females to reproduce appears only near the end of the reproductive season, when high-quality (larger) females have already mated. A reproductive opportunity for smaller females may also arise from the fact that at higher temperatures sex roles may be more equal, decreasing competition between females. For example, in *S. typhle*, higher temperatures tend to decrease differences in the potential reproductive rates of males and females (Ahneshjo 1995).

Different-sized females may thus adopt distinct reproductive tactics during the reproductive season, with large dominant females probably imposing reproduction constraints on small females. An indication of the disruption of the 'normal pattern' (larger females showing more interest and spending more time near stimulus fish) can be viewed at 24°C (end of the breeding season), where smaller females showed a significantly greater 'interest' than larger females. At high latitudes, where the breeding season tends to be shortened to a few months per year, less attractive females of a sexual reversed syngnathid species,

S. typhle, reallocate their resources from present to future reproduction (Berglund 1991). Nevertheless, in warmer waters where the breeding season is considerably longer, such as that of *S. abaster* in Portugal (K. Silva unpublished data), inferior quality (smaller) females may be able to avoid postponing reproduction to the next breeding season since males might still be able to receive eggs near the end of the breeding season (see also Jones et al. 1999). In *N. lumbriciformis*, the number of eggs per breeding male was found to be significantly higher during the last months of the breeding period when compared with the onset and middle of the reproductive season. Monteiro et al. (2006) interpreted this result as an alteration of the egg laying strategy, suggesting that in this cryptic pipefish there also seems to be a modification of some reproductive parameters occurring at the end of the breeding season.

There are, however, alternative hypotheses that would explain the same pattern. Regardless of sex-role reversal, in fish, female fecundity tends to increase with size (e.g. Berglund et al. 1986; Kraak & Bakker 1998; Herdman et al. 2004). If the breeding season is also the main time window when fish can feed and grow, small females may be forced to allocate a fraction of the reproductive season to growth, limiting spawning to a shorter period before breeding ceases. It is important to note, however, that the two hypotheses are not mutually exclusive: it might well be that both competition by larger females and the need to devote part of the breeding season to growth favour the alteration of the breeding interval in smaller individuals.

Besides contributing to a better understanding of potential sources of variability in mating behaviour, studying the effect of temperature on syngnathid behaviour and reproduction might be of great importance in a time when the prospect of global warming is of increasing concern (Walther et al. 2002).

Acknowledgments

We would like to thank everybody who helped during the laboratorial work, especially Pedro Correia and Armando Jorge, as well as Jorge Salgueiro for the review of the manuscript. We also thank Prof Anders Berglund and one anonymous referee for their suggestions and criticism that greatly improved the manuscript. Prof. Vitor Almada's participation was partially funded by Programa Plurianual de Apoio às Unidades de Investigação. Nuno Monteiro's participation was funded by Fundação para a Ciência e a Tecnologia (FCT-SFRH/BPD/14992/2004) and Programa Plurianual de Apoio às Unidades de Investigação. K.S. participation was funded by Fundação para a Ciência e a Tecnologia (FCT-SFRH/BD/13171/2003). This work was partially funded by FCT (POCI/MAR/60895/2004).

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