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When are neighbours 'dear enemies' and when are they not? The responses of territorial male variegated pupfish, *Cyprinodon variegatus*, to neighbours, strangers and heterospecifics

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Dear enemy recognition reduces the costs of territorial defence in some species, but not others, when a neighbour is more threatening to a resident's fitness than an intruder. I asked whether dear enemy effects were fixed in a particular species, or if the reduced aggression between a resident and neighbour was disrupted by the presence of potential mating opportunities. Observing variegated pupfish, *Cyprinodon variegatus*, in the field and in the laboratory, I examined the effects of a female's presence in a male's territory on residents' aggressive responses to conspecific neighbours and strangers as well as heterospecific opponents. Although reduced aggression consistent with dear enemy recognition was seen between conspecific neighbours in the absence of females, the presence of a female in a male's territory instigated comparably greater aggression between the neighbours. No reduction in aggression was seen between pupfish males and heterospecific opponents. These findings suggest that dear enemy recognition may be a flexible, rather than a fixed, feature of the relationship of neighbouring conspecific males. Despite the disruption in dear enemy recognition caused by a female, residents in the laboratory faced with neighbours spent more time associating with the female than residents faced with strangers. This allowed the residents to secure as many spawns as did males who had been faced with no competitor. Residents faced with any other type of opponent had reduced reproductive success, suggesting that the dear enemy relationship between residents and neighbours is more complex than simply a reduction in aggression.

Although territorial neighbours should represent one another's foremost competitors (Alexander 1974; Getty 1987), neighbouring residents often direct less aggression towards each other than towards nonterritorial intruders (Wilson 1975). This phenomenon of diminished aggression to stable neighbours relative to intruding strangers is termed 'dear enemy' recognition (Fisher 1954) and has been observed in a number of territorial species, including mammals (e.g. Barash 1974), amphibians (e.g. Jaeger 1981), birds (e.g. Armstrong 1991), reptiles (e.g. Qualls & Jaeger 1991), fish (e.g. Leiser & Itzkowitz 1999) and insects (e.g. Pfennig & Reeve 1989).

One hypothesis proposed to explain the occurrence of dear enemy recognition has emphasized the relative threats that neighbours versus strangers pose to a territorial resident (Temeles 1994). Consistent with theoretical game models (e.g. Maynard Smith & Parker 1976; Maynard Smith 1982), this hypothesis predicts that a resident should invest more in aggression against a more threatening individual that is capable of inflicting greater losses on the resident (Getty 1987). Presumably, prolonged, escalated aggression should not occur between neighbours inasmuch as neighbours, that already possess territories, are not threatening to each other's territorial resources (Wilson 1975; Jaeger 1981). Contrastingly, nonterritorial intruders are often relentlessly attacked by residents (Parker 1974; Riechert 1979; Enquist & Leimar 1987; Stamps & Krishnan 1997), because intruders may be threatening as potential usurpers of territorial space (Getty 1981, 1987).

The relative threats of neighbours versus strangers also appear to explain circumstances under which dear enemy recognition does not occur. When neighbours are more threatening than strangers to a resident, the resident should not show diminished aggression towards neighbours compared with strangers. For example, Temeles (1989, 1990) found that territorial northern harriers, *Circus cyaneus*, are more aggressive to neighbours than to floaters because floaters intrude to steal food, whereas

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neighbours attempt not only to steal food but also to usurp portions of the residents' territories. Ferkin (1988) found that resident male meadow voles, *Microtus pennsylvanicus*, respond with less aggression towards strangers than neighbours because strangers are often transient males, whereas neighbours compete for females within each other's territories, usurp portions of each other's territories and eat each other's offspring.

Several empirical studies have documented the presence of dear enemy recognition between neighbours of a territorial species (e.g. Krebs 1982; Fox & Baird 1992; Heinze et al. 1996) or its absence (e.g. Beletsky 1983; Temeles 1990; Speirs & Davis 1991), but there has been little investigation within a species of whether changing social conditions may change the relative threats of neighbours versus strangers and the relative responses of residents to these threats. The lower threat of a neighbour may free a resident to engage in other behaviours, such as fights with intruders as in convict cichlids, *Archocentrus* (formerly *Cichlasoma*) *nigrofasciatum* (Leiser & Itzkowitz 1999). Under these circumstances, the presence of a neighbour should be expected to free a resident for courtship and mating behaviours with available females, whereas the presence of an intruder, who is attempting to steal matings from the resident (e.g. Howard & Young 1999), should cause the resident to forego mating opportunities in lieu of defence. Alternatively, dear enemy recognition may be important in reducing aggression between neighbours in the absence of potential mates (i.e. when neighbours do not directly threaten the fitness of the resident), but may be disrupted if a neighbour can usurp reproductive opportunities from the resident (Ferkin 1988; Howard & Young 1998). Under these conditions, a neighbour may be as threatening to the resident as an intruder, and the reduced aggression between neighbours characteristic of dear enemy recognition may not be observed.

I examined interactions of territorial male variegated pupfish, *Cyprinodon variegatus*, with neighbouring and intruding conspecific and heterospecific males under field and laboratory conditions to determine whether the relative threats of and the amount of aggression shown by residents to these opponents are altered by changes in the availability of a potential mate. This pupfish, as well as a number of its congeners (*C. pecosensis*, Kodric-Brown 1983; *C. atrorus*, Itzkowitz & Minckley 1969; *C. bifasciatus*, Kodric-Brown 1977, 1978; *C. macularius*, Barlow 1958) has a promiscuous breeding system centred around territorial defence by adult males. Territorial males often establish themselves in a cluster. Females swim through the cluster and may spawn one or more times with one to several males daily, laying eggs in multiple territories. Males may mate with several females daily. Territorial neighbours frequently interact, and intrusions into territories by strange males are common (Itzkowitz 1974, 1981). Both neighbours and strangers may interrupt the spawning sequence of a resident male and a female (Itzkowitz 1974); spawning interruptions often result in the female leaving the territory to deeper water or crossing into the territory of the neighbour that had interrupted the spawn (Itzkowitz 1974). Therefore, a male's reproductive success seems to be closely related to the male's ability to exclude not only strange males but also neighbours from his territory.

In addition to defence against conspecific opponents, resident *C. variegatus* males attack heterospecific intruders, including poecilids (e.g. *Gambusia* sp.) and killifishes (e.g. *Fundulus* sp.). These species prey on pupfish eggs, consuming eggs within the territories of resident males. The heterospecifics have not been observed interrupting spawns (Itzkowitz 1974), and pupfish males defer attacking heterospecifics with regard to conspecific opponents. However, as egg predators, heterospecifics also pose a potential threat to the fitness of residents.

To examine the nature of dear enemy recognition in *C. variegatus*, I first considered how territorial males responded to neighbours, intruders and heterospecific fish in the absence of females. Based on dear enemy recognition (Temeles 1994), I predicted that a territorial male would show the greatest aggression towards a stranger, followed by a neighbour and a heterospecific opponent, respectively. In the absence of a potential mate, all three of these competitors could potentially pose a threat to a resident male by preying upon eggs present in the resident's territory (Kodric Brown 1986), whereas intruders and neighbours may pose a threat as usurpers of the resident's entire territory or a portion of it, respectively. I did not expect to see diminished aggression towards a neighbouring heterospecific compared to a strange heterospecific male, as heterospecific opponents should continue to represent a threat to the residents' fitness, regardless of exposure to the resident. That is, I expected dear enemy recognition to be a species-specific phenomenon.

The dear enemy relationship of diminished aggression between a resident and a conspecific neighbour should, in turn, influence the manner in which residents interact with females. I predicted that residents faced with a conspecific neighbour would spend more time courting and spawning with an available female than residents faced with a conspecific stranger. However, the presence of females has been shown to instigate aggression in male *C. variegatus* (Itzkowitz 1977). In this instance, I predicted that a female's presence would be disruptive to dear enemy effects, resulting in heightened aggression between the resident and neighbour and causing the resident to focus on territorial defence rather than on courtship and spawning.

In contrast to having a dear enemy neighbour, I predicted that territorial residents faced simultaneously with a female and a strange male would opt to defend their territories rather than court and spawn with the female. Studies have shown that territorial intrusions by male competitors are common in *Cyprinodon* (Itzkowitz 1974), and residents may often lose reproductive opportunities to intruding males (Itzkowitz 1974; Kodric-Brown 1981, 1986, 1988). Alternatively, the presence of nonterritorial males may be attractive to females (Hill 1991). For instance, Kodric-Brown & Nicoletto (1993) suggested that resident males with the greatest number of competitors would have relatively high mating success despite previous observations that these males engaged in greater numbers of aggressive interactions (Kodric-Brown 1978, 1983). In this case, the reproductive success of residents faced with a stranger should be as high as that of residents faced with a neighbour.

Unlike conspecific opponents, the relative threat of heterospecifics should not be expected to change in the absence versus presence of a female, because these fish are likely to consume any eggs found in a male's territory, regardless of when they were laid.

METHODS

Field Study

I studied a population of variegated pupfish, *C. variegatus*, in a small brackish pond, approximately 50 m in diameter, located at Bombay Hook National Wildlife Refuge, Smyrna, Delaware, U.S.A. This pupfish occurs commonly in tidal pools and estuaries along the eastern coast of the U.S.A.; it ranges from Cape Cod, Massachusetts to southern Florida, the Gulf of Mexico and the West Indies. The breeding system of *C. variegatus* depends on climate; in the Mid-Atlantic U.S.A., these fish breed when water temperatures are warm (24–30 °C; Itzkowitz 1974). The breeding season typically extends from middle to late April through to September (Itzkowitz 1974).

The mating system of this species centres around the defence of territories by males. Territories are defended by aggressive behaviours including chases, lateral displays and boundary fights (Itzkowitz 1974); territorial neighbours, nonterritorial conspecifics and heterospecifics are often attacked by territorial residents. Although lateral displays and boundary fights (culminating in the males' tailbeating each other) do occur, these behaviours are relatively rare compared with chases (Itzkowitz 1974). Females often enter and leave a given male's territory. Mating sequences begin as a female enters a territory; she will either be immediately courted and spawn with a male, or she will momentarily 'wait' for courtship before leaving. Spawning events are brief, with the pair descending to the substratum and developing a sigmoid position. Females typically lay one demersal egg per spawn (Kodric-Brown 1986). Spawning interruptions by male competitors are common (Itzkowitz 1974).

I conducted observations on 17 different territorial males during August 2000. Each male was observed during late morning hours (1000–1200 hours) from a position on shore for 15 min. This period of time was sufficiently short to allow for a number of males to be watched daily but sufficiently long to provide an accurate estimate of the males' territorial behaviour and was considered appropriate because it reflected the median amount of time that territorial pupfish had been viewed by previous researches either in the laboratory or in the field (mean of 17.2 min, range 5–30 min: Raney et al. 1953; Itzkowitz 1974, 1977, 1981, 1984; Kodric-Brown 1977, 1978, 1988; Leiser & Itzkowitz, in press). During the 15 min, I recorded into a notebook the behaviours in which residents engaged, in the sequence in which they were observed. The aggressive behaviours that were recorded included chases, lateral displays and bouts of tailbeating. Initially, I analysed aggressive data separately to determine whether dear enemy effects might involve not only the number of aggressive behaviours but also the type of behaviours displayed by residents towards neighbours relative to intruders. In addition to recording the males' behaviour, I noted the identity of the opponent (i.e. conspecific neighbour, conspecific intruder, heterospecific intruder). Heterospecific intruders included *Fundulus majalis* and *Gambusia affinis*, both of which are presumed *Cyprinodon* egg predators (Itzkowitz 1974). Additionally, I recorded the number of spawns for each resident male. Spawns were distinguished as successful or interrupted. Successful spawns ended with a characteristic jerking movement of the male and female. Interrupted spawns often did not include the jerk, and the male would frequently leave the female to chase the interrupting male. If spawns were interrupted, I recorded the identity of the interrupting fish.

I divided the total number of aggressive interactions (including chases, displays, tailbeats and boundary fights) that residents displayed towards neighbours, intruders, or heterospecifics into two categories: (1) the proportion of interactions that residents had with each type of opponent immediately before or after the resident had interacted with a female (either via courtship or spawning behaviours) and (2) the number of interactions the residents had with the opponents in the absence of a female (i.e. when a female was not in the territory). I analysed aggressive data with one-way within-subject analyses of variance (ANOVAs) using the statistical software package STATISTICA (StatSoft 1998). Prior to analysis, the percentage data were arcsine transformed to meet assumptions of parametric analyses (Zar 1999) and the data on the number of times that residents chased opponents were square-root transformed to meet normality assumptions (Kolmogorov–Smirnov test: d=0.120, N_h=16, NS). I conducted effect size and power estimates following Keppel (1991).

Laboratory Study

Subjects

The fish used in this study were similar-sized adult male and female variegated pupfish, *C. variegatus*, and male striped killifish, *F. majalis*, that were collected from either a large tidal pool in Point Lookout State Park, Scotland, Maryland, U.S.A., or a tidal pool and stream delta on the property of Horsehead Wetlands Center, Grasonville, Maryland. After transfer to the laboratory, the fish were allowed to acclimate to freshwater conditions for ease of study. All fish were maintained on a 14:10 h light:dark cycle at 22 ± 2 °C. The pupfish were housed in single-sex groups. Males were kept at relatively high density (one fish/3 litres) in either 473-litre or 220-litre stock aquaria, while females were housed at lower densities (one fish/7.5 litres) in 37.5- or 75-litre stock aquaria. *Fundulus* were maintained in a 473-litre stock aquarium that contained a mixed-sex population of pupfish not used in the experiments. All fish were fed daily ad libitum with commercial flake food or shrimp pellets. Approximately 350 fish were available for the experiments.

Table 1. Series of replicates conducted in laboratory study of *C. variegatus*

Contestants (series type)	*N*
1 Resident alone (male alone)	16
2 Resident–Conspecific stranger	16
3 Resident–Conspecific neighbour	16
4 Resident–Heterospecific stranger	12
5 Resident–Heterospecific neighbour	12
6 Pair alone (male–female alone)	20
7 Pair–Conspecific stranger	22
8 Pair–Conspecific neighbour	19
9 Pair–Heterospecific stranger	16
10 Pair–Heterospecific neighbour	16

Experimental design

Experimental aquaria, each 75 litres in total volume (measuring 30 × 75 cm; water depth 20 cm) with a thin layer (5 ± 2 mm) of fine sand substratum (see below), were divided into three equally sized compartments using one transparent and one opaque partition; the side with the opaque partition was randomized. Each compartment measured 30 × 25 cm. The outside bottom of each aquarium was marked every 5 cm, dividing the aquarium into 15 regions. One air stone was placed in the central rear of each aquarium. Between replicates, the aquaria were drained, cleaned and refilled. The sand used as substratum was all-purpose, sterilized sand that had been purchased from a local hardware retailer. Prior to the experiments, the sand was sifted through an archaeological sieve with a 500-m pore diameter. Only the sand that passed easily through the sieve was retained for use in the experiments (i.e. granule size ≤500 m).

Replicate series

I staged 10 series of replicates in which the number and category of individuals in the aquaria differed (Table 1).

Residents. In each series, I selected one male pupfish at random from a male stock aquarium, measured his total length to the nearest 0.1 mm, and placed him into the central compartment of an experimental aquarium. I allowed the male to acclimate to the aquarium for 24 h and termed him the ‘resident’. Residents ranged in size from 32.9 to 57.1 mm. In one series, I observed the resident alone (‘resident alone’, *N*=16); in the remaining series I considered the residents’ responses to other individuals. Following use, I returned residents to the male stock aquarium from which they had been selected. Although the males may have been used more than once throughout the course of the experiment, care was taken to ensure that no males (including neighbours and strangers, see below) were used more than once per month, allowing adequate adjustment to stock conditions before being used again. In addition, the number of males available during the experiment made it unlikely that males were used as the same category of individual twice.

Females. Five of the 10 series of replicates (Table 1) involved a female’s interaction with the resident male. Seventy-two hours prior to introducing the resident into the experimental aquarium, I selected a female at random from one of the female stock aquaria. I measured the total length of the female and isolated her in half of a 37.5-litre aquarium. Females ranged in size from 33.8 to 53.2 mm. Females and residents were as close in size as possible and were no more than 2.1 mm in total length different during any of the interactions. Following the isolation period, I introduced the female into the experimental aquarium simultaneously with the resident. I placed the female in the side compartment, separated from the central compartment by the opaque partition. I then allowed the female to acclimate to the experimental aquarium for 24 h. Although not the most natural situation, this methodology seemed to provide as little disturbance to the females as possible immediately prior to beginning the replicate, thus ensuring that a greater proportion of females would spawn over other methodologies (personal observation). In one series of replicates, the resident and female were the only fish in the experimental aquarium (‘experimental pair alone’, *N*=20). The remaining series involved either a neighbour (‘experimental pair–neighbour’) or stranger (‘experimental pair–stranger’). Females were used only once throughout the course of the experiment.

Neighbours. In the series using neighbours, I selected a male from a respective conspecific or heterospecific stock aquarium simultaneously with the resident, measured the males, and introduced the conspecific or heterospecific male into the side compartment of the experimental aquarium behind the transparent partition. Residents and neighbours were as close in total length as possible and were no more than 1.6 mm different (for conspecific neighbours) or 3.0 mm different (for heterospecific neighbours). The neighbour was allowed to acclimate to the aquarium and to interact visually with the resident for 24 h. Two series of replicates with neighbours involved females (‘experimental pair–conspecific neighbour’, *N*=19; ‘experimental pair–heterospecific neighbour’, *N*=16); the other two did not (‘male–conspecific neighbour’, *N*=16; ‘male–heterospecific neighbour’, *N*=12).

Strangers. The remaining series involved a male selected randomly from one of the stock aquaria and introduced into an experimental aquarium behind the transparent partition following the 24-h acclimation period of the resident. This male, termed a ‘stranger’, was similar in size to the resident (no more than 1.0 mm in total length different in all interactions with conspecific strangers or 3.3 mm for heterospecific strangers). As with interactions with neighbours, the stranger series either involved the female (‘experimental pair–conspecific stranger’, *N*=22; ‘experimental pair-–heterospecific stranger', N=16) or did not ('male–conspecific stranger', N=16; 'male–heterospecific stranger', N=12).

Data collection and analyses

Following the 24-h acclimation period, I removed the opaque partition and videotaped the aquaria for 30 min. Immediately after the videotaped observation session, I removed all of the fish from the aquarium and returned them to their appropriate stock aquaria. In replicates involving a female, I siphoned the sand substrate of the experimental aquarium through an archaeological sieve with an 800-m pore diameter. This allowed the sand to pass, while any eggs were retained in the sieve. I counted the eggs and transferred them to a 4-litre glass jar filled with water and outfitted with an air stone. Most eggs were fertilized and hatched.

During the 30-min observation period, I recorded the positions of the resident and female (when present) every 15 s in relation to which of the 15 regions each fish was located. I recorded the position of each fish as the location of the tip of its snout. From the 120 positional data points collected for the resident, I recorded the time that the resident spent confronting its opponent as the number of periods in which the resident was observed to be within 5 cm of the transparent partition. When both the resident and female were present, I recorded the number of time periods that the two were seen together (i.e. in the same region of the aquarium) and away from the transparent partition (>5 cm distant). I used these latter data to assess the duration that the resident and female associated with one another but not with either the neighbour or the stranger. I also recorded the number of courtship displays the resident performed towards the female. A courtship display occurred when the male positioned himself parallel to the female, placed the tip of his snout slightly behind her operculum and contacted her with his trunk (Itzkowitz 1974). Displays often occurred immediately before a female spawned, but did not always result in a female's spawning. I recorded all spawning bouts, which also corresponded to the number of eggs laid, as female pupfish lay only one egg per spawning event (Kodric-Brown 1977, 1981, 1988; Itzkowitz 1978). I used a Pearson product–moment correlation (Keppel 1991) to examine whether females spawned in proportion to their body sizes, as expected in fish. To examine the possible positional effects that the respective opponents had on a female's spawning, I recorded the locations at which eggs were laid with respect to the partition and analysed these data using one-sample t tests (Keppel 1991). The data for the position of residents' confronting their opponents were analysed using a two-way unweighted means analysis of variance, ANOVA (Keppel 1991). I analysed both the male–female positional and courtship data with one-way unweighted means ANOVAs and the proportion of females that spawned across the series of replicates using chi-square tests (Zar 1999).

I also recorded data on the aggressive responses of the resident to his competitors from the videotape. These data included the number of times the resident attempted to bite a competitor through the transparent partition (resulting in contact of the resident's mouth with the partition), the number of times the resident 'charged' at an opponent (by swimming from a position away from the partition rapidly towards the partition, contacting it), and the number of times the resident performed lateral displays to a competitor. A lateral display was noted when the resident, while adjacent to the partition, oriented himself parallel to the partition and fanned his unpaired fins while hovering. Due to the presence of the partition, I could not distinguish between lateral displays and tailbeats; therefore, I combined these behaviours for analyses. I analysed the data using a three-way unweighted means ANOVA. Data analyses were performed using the statistical packages DATASIM (Version 1.2, STATISTICA, StatSoft 1998). The data were transformed by the equation $X'=\sqrt{(X+0.375)}$ (Zar 1999) to meet normality assumptions (Kolmogorov-Smirnov test: d=0.08, NS). Due to the unequal sample sizes for the experimental series, I conducted effect size (ω^2) and power estimates ($1-\beta$) with respect to the harmonic sample size (N_h=16) (Keppel 1991). This allowed for consistency with the unweighted means ANOVAs.

RESULTS

Field Study

Males defending territories ranged in size from 3.0 to 4.5 cm ($\bar{X}$=3.8, N=17) and defended portions of silt substrate in shallow water, less than 25 cm deep. Each resident observed had at least one neighbour ($\bar{X}$=1.5 neighbours). The majority of males (76.5%) spawned successfully with at least one female.

Territorial males spent much of the 15-min observation period attacking individuals that crossed into their territories. Aggressive defence of territories focused on conspecific neighbours and intruders as well as on heterospecific opponents, including *F. majalis* and *G. affinis*. Resident males did not chase all opponents equally (one-way within subject ANOVA: $F_{2,32}$=74.77, P<0.05, ω^2=0.74, $1-\beta$>0.99; Fig. 1a). That is, residents chased conspecific intruders ($\bar{X}\pm$SE=17.76 ± .61) significantly more times than conspecific neighbours (3.35 ± 0.94; ad hoc pairwise contrast within ANOVA: $F_{1,16}$=83.83, P<0.05, ω^2=0.62, $1-\beta$>0.99) and chased conspecific neighbours significantly more times than heterospecific opponents (1.18 ± 0.32; ad hoc pairwise contrast within ANOVA: $F_{1,16}$=5.75, P<0.05, ω^2=0.09, $1-\beta\approx$0.55). The overall numbers of times that residents displayed to or engaged in tailbeats with opponents were low and consequently combined for analysis. Although resident males chased conspecific intruders more than neighbours, residents engaged in similar numbers of displays and tailbeats towards conspecific intruders (1.65 ± 0.33) and neighbours (1.24 ± 0.39; one-way within-subject ANOVA: $F_{1,16}$=0.50, NS; Fig. 1b). Residents never displayed to or engaged in tailbeats with heterospecific opponents; thus, analyses of these data excluded heterospecifics.

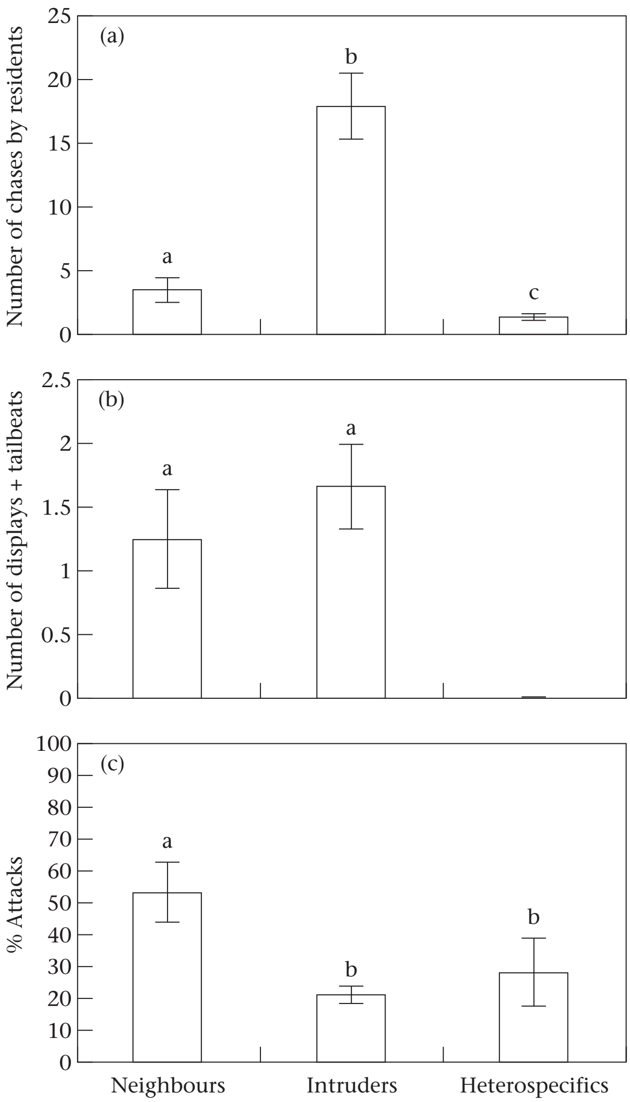

Figure 1. Means (±SEs) for (a) the number of times residents chased conspecific neighbours and intruders and heterospecific intruders, (b) the number of times residents displayed to or engaged in tailbeats with conspecific neighbours or intruders and (c) the percentages of the number of total attacks directed against each type of opponent while a female was present on the male's territory during the 15-min observation session. Different letters indicate significant differences in (a) (one-way within-subject ANOVA: $P<0.05$) and (c) (one-way within-subject ANOVA: $P<0.05$). No significant difference was found in the number of displays and tailbeats (one-way within-subject ANOVA: NS).

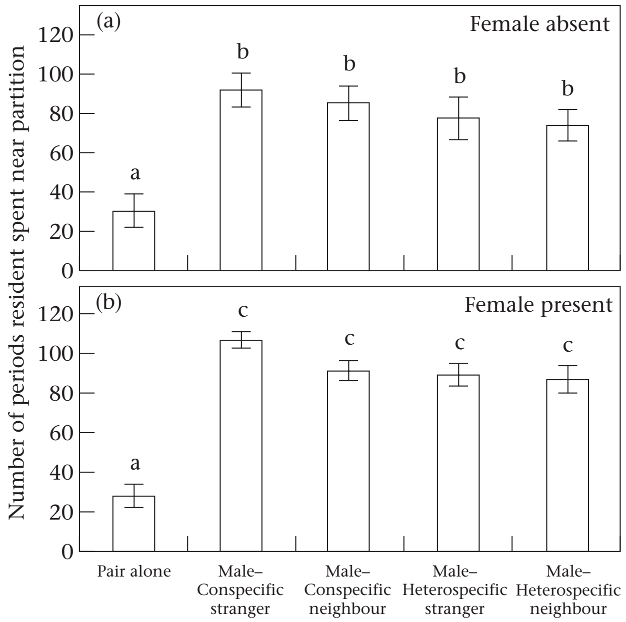

Figure 2. Means (±SEs) for the number of 15-s time periods that residents were observed within 5 cm of the transparent partition during the 30-min observation session, confronting an opponent in (a) the absence or (b) the presence of a female. Different letters indicate significant differences (two-way unweighted means ANOVA: $P<0.05$).

The presence of a female in a resident's territory influenced the amount of aggression that residents showed towards specific opponents. Although residents attacked conspecific neighbours less than intruders, a greater proportion of the total number of interactions with neighbours (chases+displays+tailbeats) occurred immediately before or after interactions with females (52.89 ± 9.23%) than the proportion of interactions with intruders in the presence of females (20.64 ± 2.48%; ad hoc pairwise contrast within ANOVA: $F_{1,16}=13.48$, $P<0.05$, $\omega^2=0.20$, $1-\beta\approx0.80$; overall one-way within-subject ANOVA: $F_{2,32}=4.32$, $P<0.05$, $\omega^2=0.12$, $1-\beta\approx0.60$; Fig. 1c). The proportion of attacks on heterospecific opponents in the presence of females (27.94 ± 10.48%) was similar to the proportion of attacks on intruders (ad hoc pairwise contrast within ANOVA: $F_{1,16}=0.47$, NS).

Laboratory Study

The average number of time periods that residents spent near the transparent partition differed between replicate types. Both the main effects of female absence versus presence (two-way unweighted-means ANOVA: $F_{1,155}=4.53$, $P<0.05$, $\omega^2=0.02$, $1-\beta<0.40$) and the main effect of opponent (neighbour versus stranger; two-way unweighted-means ANOVA: $F_{4,155}=32.45$, $P<0.05$, $\omega^2=0.08$, $1-\beta\approx0.55$) were significant. The female by opponent interaction was not significant (two-way unweighted-means ANOVA: $F_{4,155}=0.59$, NS; Fig. 2a, b). There was no difference in the amount of time residents spent near the partition when faced by any opponent in the absence of the female (Scheffé test: $F_{1,155}=2.98$, NS; Fig. 2a) or in the presence of a female (Scheffé test: $F_{1,155}=2.938$, NS; Fig. 2b). The average amount of time that residents spent at the partition when alone and with only a female in the aquarium (pair alone) did not differ (Scheffé test: $F_{1,155}=0.06$, NS), but were significantly lower than any of the series of replicates involving competitors (Scheffé test: $F_{1,155}=6.07$, $P<0.01$, $\omega^2=0.42$, $1-\beta\approx0.98$). That is, residents spent little time at the partition when no competitors were in the aquaria. Comparatively, residents spent more time at the partition when females were absent but opponents were present,

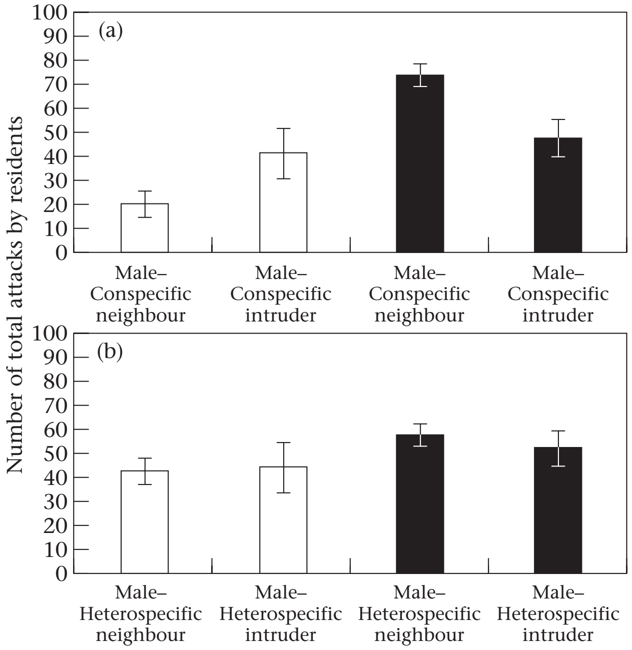

Figure 3. Means (±SEs) for the number of times residents attacked (a) conspecific versus (b) heterospecific opponents in the absence (□) and presence (■) of a female during the 30-min observation period. See text for discussion of three-way unweighted means ANOVA and comparisons.

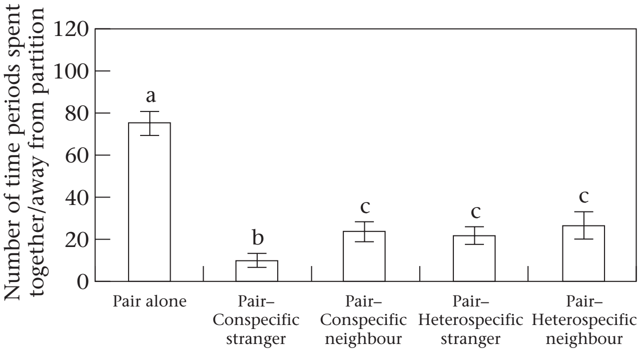

Figure 4. Means (±SEs) for the number of 15-s time periods during the 30-min observation session that residents and females were observed to be together and away from the transparent partition. Different letters indicate significant differences (one-way unweighted means ANOVA: $P<0.05$).

and spent the greatest amount of time near the partition when both a female and an opponent were present.

As in the field, residents in the laboratory performed no displays or very few displays towards heterospecific opponents. Also similar to the field results, the number of displays that residents performed towards conspecific opponents was low, and there were no qualitative differences in the number of displays and tailbeats that residents performed towards conspecific neighbours or strangers. Therefore, I combined these data with the number of charges and bites for analysis. The total number of attacks (i.e. mean number of times residents charged at, lateral-displayed towards and attempted to bite their opponents) was dissimilar across replicate types (Fig. 3a, b). In the three-way unweighted means ANOVA, the main effect of female absence versus presence was significant ($F_{1,121}=17.81$, $P<0.05$, $\omega^2=0.12$, $1-\beta\approx0.84$), but neither the main effect of opponent ($F_{1,121}=0.04$, NS) nor the main effect of species was significant ($F_{1,121}=3.31$, NS). The female by opponent interaction ($F_{1,121}=3.44$, NS), the opponent by species interaction ($F_{1,121}=0.37$, NS) and the three-way interaction ($F_{1,121}=2.57$, NS) were also not significant. However, there was a significant female by species interaction ($F_{1,121}=3.92$, $P<0.05$, $\omega^2=0.02$, $1-\beta<0.40$), indicating that residents responded to the different species of opponents differently in the absence versus presence of a female. Residents did not attack heterospecific neighbours and strangers differently, regardless of the presence (56.59 ± 4.81 for neighbours, 51.25 ± 5.63 for strangers) or absence (42.08 ± 4.62 for neighbours, 43.25 ± 7.15 for strangers) of a female (Scheffé test: $F_{1,121}=2.21$, NS; Fig. 3b). However, consistent with predictions of dear enemy recognition, in the absence of a female, there was a significant trend for residents to attack conspecific neighbours (19.81 ± 5.32) less than conspecific strangers (40.56 ± 10.34; ad hoc pairwise contrast: $F_{1,121}=3.84$, $P=0.05$, $\omega^2=0.02$, $1-\beta<0.40$; Fig. 3a), although this difference represented a relatively small effect (Keppel 1991). Contrary to expectations based on fixed dear enemy effects, this tendency was reversed for conspecific neighbours (72.26 ± 11.38) compared to strangers (46.18 ± 4.15) in the presence of a female, although not significant (ad hoc pairwise contrast: $F_{1,121}=3.05$, $P=0.08$). Residents did attack conspecific neighbours significantly more times in the presence of a female than in the absence of a female (ad hoc pairwise contrast: $F_{1,121}=26.06$, $P<0.05$, $\omega^2=0.16$, $1-\beta\approx0.70$; Fig. 3a). This difference was not present for conspecific strangers (ad hoc pairwise contrast: $F_{1,121}=2.25$, NS; Fig. 3a).

During the five series of replicates in which residents interacted with females (i.e. experimental pair alone; experimental pair–conspecific stranger; experimental pair–conspecific neighbour; experimental pair–heterospecific stranger; experimental pair–heterospecific neighbour; Table 1), the total number of time periods that the resident male and female were seen together and away from the transparent partition were significantly different, depending on interaction type (one-way unweighted means ANOVA on $X'=\log(X+1)$ transformed data (Zar 1999); Kolmogorov–Smirnov test: $d=0.095$, $N_h=16$, NS): $F_{4,88}=19.16$, $P<0.05$, $\omega^2=0.48$, $1-\beta>0.99$; Fig. 4). When resident males and females were the only fish in experimental aquaria, the pair was, on average, observed together and away from the partition significantly more times (75.0 ± 5.49) than when resident males were confronted by an opponent (ad hoc multiple contrast within one-way ANOVA: $F_{1,88}=49.06$, $P<0.05$, $\omega^2=0.38$, $1-\beta>0.99$). Resident males and females associated with one another significantly more in the presence of a neighbouring conspecific male (23.32 ± 4.38) than in the presence of a strange conspecific male (9.68 ± 3.24; ad hoc pairwise contrast: $F_{1,88}=14.10$, $P<0.05$, $\omega^2=0.86$, $1-\beta>0.99$). However, the mean number of times that

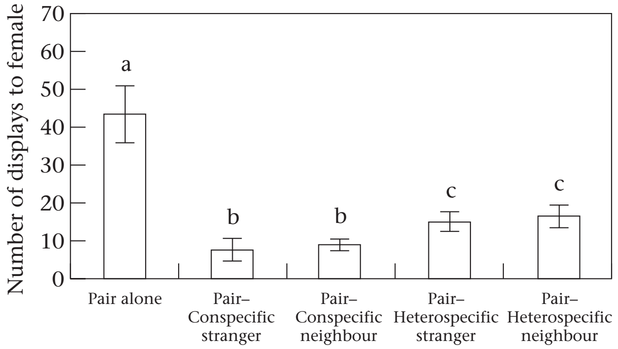

Figure 5. Means (±SEs) for the number of times that residents performed lateral displays towards females. Different letters indicate significant differences (one-way unweighted means ANOVA: $P<0.05$).

resident males and females spent together and away from the partition did not differ for heterospecific neighbours (26.81 ± 6.05) and heterospecific strangers (21.56 ± 3.92; ad hoc pairwise contrast: $F_{1,88}=0.12$, NS). There was also no difference in the amount of association between resident males and females in the presence of heterospecific opponents and conspecific neighbours (Scheffé test: $F_{1,88}=0.10$, NS).

Despite the observation that residents spent more time associating with females in the presence of conspecific neighbours than conspecific strangers, the number of times that residents displayed to females for these two types of interactions did not differ (ad hoc pairwise contrast within one-way ANOVA conducted on data transformed by $X'=\sqrt{(X+0.375)}$ (Zar 1999): $F_{1,88}=1.18$, NS). In contrast, when experimental pairs were isolated, the number of displays that residents directed towards females (43.35 ± 7.50) was significantly greater than when either conspecific strangers (7.50 ± 2.86), conspecific neighbours (8.53 ± 1.39), heterospecific strangers (14.69 ± 2.36), or heterospecific neighbours (16.56 ± 2.94) were present (one-way ANOVA: $F_{4,88}=19.87$, $P<0.05$, $\omega^2=0.49$, $1-\beta>0.99$; Fig. 5). However, the number of displays was significantly greater for heterospecific opponents compared with conspecific opponents (Scheffé test: $F_{1,88}=11.29$, $P<0.01$, $\omega^2=0.11$, $1-\beta<0.50$).

Of the 93 females used in the interactions, 33 spawned with resident males. The number of eggs laid by spawning females was proportional to female body length (Pearson's product–moment correlation: $r_{31}=0.51$, $P<0.01$; Fig. 6), as expected in fish. The relative proportions of eggs laid adjacent to the partition were similar across the series (one-way ANOVA: $F_{4,28}=1.86$, NS) and did not differ from what would be expected if females laid eggs randomly throughout the territory (i.e. =10% of the eggs were laid within 5 cm of the partition; $\bar{X}\pm\text{SE}=16.17\pm4.28$; one-sample t test: $t_{32}=1.44$, NS; Keppel 1991). Therefore, rather than comparing the number or location of spawns received by residents, I analysed the proportion of females spawning across series types. The number of females that spawned with residents differed across the five series involving females (chi-square test: $\chi^2_4=21.03$, $P<0.001$; Fig. 7). Comparisons within the chi-square analysis indicated no significant difference between the proportion of females spawning with residents when the pair was alone (60%) and in the presence of neighbours (63.2%; $\chi^2_1=0.04$, NS). The proportion of females spawning was also similar in the presence of conspecific strangers (9.1%), heterospecific strangers (18.8%) and heterospecific neighbours (25%) (comparison within the chi-square analysis: $\chi^2_1=1.53$, NS). The average of the pair alone and conspecific neighbour conditions was significantly higher than the average of the other three conditions (comparison within the chi-square analysis: $\chi^2_1=19.92$, $P<0.0001$).

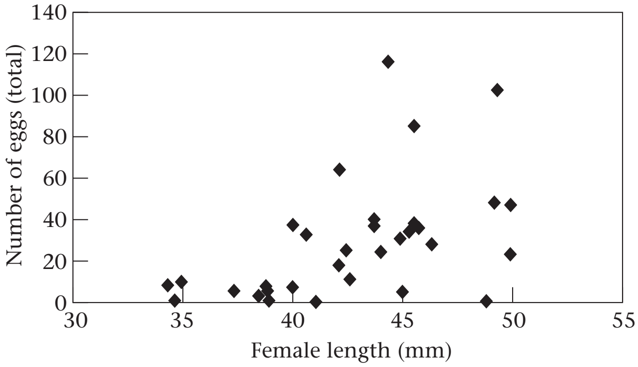

Figure 6. Relationship between total body length and the number of eggs laid by the 33 females that spawned in the different types of interactions (Pearson's product–moment correlation: $P<0.001$).

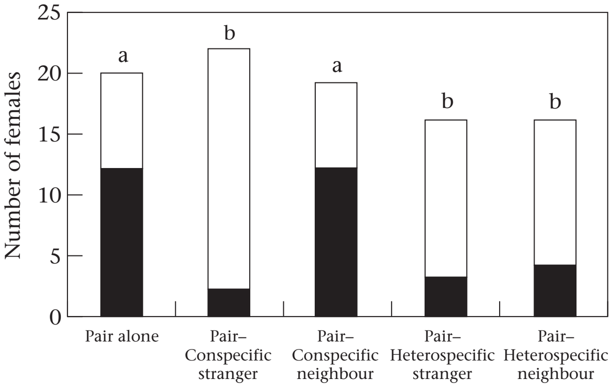

Figure 7. Relative proportions of females spawning (■) versus not spawning (□) for the five types of interactions involving residents and females. Different letters indicate significant differences (chi-square test: $P<0.001$).

DISCUSSION

In the absence of females, territorial residents directed less aggression towards neighbours relative to intruders both in the field and in the laboratory, although this trend was only marginally significant in the laboratory. These results are consistent with the prediction that dear enemy recognition should occur when neighbours are less of a potential threat to residents than are intruding strangers. Residents should be expected, on the basis of game-theoretical costs and benefits (Maynard Smith

1982; Getty 1987), to focus aggression on potential territorial usurpers as these males may be capable of inflicting greater costs on residents if they are successful in ousting a resident from its territory. The hypothesis that residents should respond to competitors based on their relative threats was also supported in the field by the residents' low number of attacks on heterospecifics. Although both conspecific neighbours and intruders as well as heterospecific fish may consume the eggs present in a male's territory (Kodric-Brown 1986), heterospecific intruders are not a threat to the resident's possession of the territory; the potential costs of the presence of these fish are lower than the potential costs posed by neighbouring or intruding conspecifics, respectively.

The data did not support the hypothesis of dear enemy recognition as a fixed feature of the relationship between neighbouring pupfish males; rather, dear enemy effects changed with changing circumstance. In the field, a greater proportion of the residents' attacks against neighbours compared with intruders occurred in the presence of a female, and in the laboratory, the resident's tendency to reduce aggression towards neighbours relative to strangers in the absence of a female was reversed in the presence of a female. In neither of these circumstances was the reduction in aggression characteristic of dear enemy recognition seen when a female was in a male's territory. This may have occurred because in pupfish, both neighbouring and intruding conspecifics may attempt to steal spawns with available females (Itzkowitz 1974; Kodric-Brown 1986). In addition to stealing spawns, however, neighbouring males may usurp peripheral portions of the resident's territory (Itzkowitz 1978; Kodric-Brown 1988) and attempt to monopolize spawning opportunities within it. The dispute over the shared territorial boundary (Kodric-Brown 1988) may be greater when a female is present, creating a situation where the resident must be more aggressive towards a neighbour in order not to lose valuable territory area and spawning opportunities (for an analogous situation in ants, see Adams 1998). In the presence of a female, a neighbour may represent a greater threat to the resident's fitness than an intruder, and the resident's responses to these opponents would appear inconsistent with those predicted by dear enemy recognition.

The prevention of interference in spawning events may explain why residents spent increased, yet similar, amounts of time confronting all opponents in the presence compared with the absence of a female. However, a greater proportion of residents faced with conspecific neighbours spawned with females than residents faced with either conspecific strangers or heterospecific opponents. In the case of conspecific opponents, the decreased reproductive success of residents faced with strangers may have been the result of their not associating with the female away from the transparent partition. Although neighbours may pose the greatest threat to a resident along the shared territorial boundary (Krebs 1971; Barlow 1974; Wilson 1975), strangers are equally threatening to the resident throughout the territory. Residents faced with neighbours spent more time associating with the female away from the boundary than did residents faced with strangers. These males achieved greater reproductive success despite performing a similar number of displays to females as residents that faced strangers.

Although residents faced with either heterospecific neighbours or strangers spent similar amounts of time associating with females compared to residents faced with conspecific neighbours, few females spawned with residents in the presence of heterospecific opponents. This occurred despite residents' courting females more in the heterospecific treatments than in the conspecific treatments. This may be explained by the relative threats posed by heterospecifics to a resident male and female. To the resident male, heterospecifics threaten to consume some of the eggs laid by the female in the territory; however, it is unlikely that they would find all of the eggs buried in the substratum of a male's territory, and these fish do not represent a threat to the male's possession of the territory. To the female, heterospecifics represent a threat to the majority of the few eggs she may lay while spawning with the male. The loss of these few, relatively large eggs (Kodric Brown 1986) to predators may be more costly to the female than to the male.

Dear enemy recognition appears to be important in competition between variegated pupfish, but the reduced aggression was not a fixed feature in resident–neighbour interactions. Although relatively lower aggression was seen in the absence of females, the presence of a female in a resident's territory incited greater aggression between the resident male and his neighbour. That is, a female's presence in a male's territory was disruptive to the dear enemy relationship of neighbours. Despite this increase in aggression, residents faced with neighbours had greater reproductive success than residents faced by other types of opponents. These results suggest that the relationship a territorial resident has with neighbours compared with intruders is a complex and dynamic one, changing relative to the circumstances in which a resident finds himself.

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