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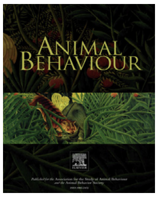

Ectoparasites modify escape behaviour, but not performance, in a coral reef fish

Sandra A. Binning[a,b,*], Jeffrey I. Barnes[c], Jaclyn N. Davies[d], Patricia R. Y. Backwell[a], J. Scott Keogh[a], Dominique G. Roche[a,b]

[a] *Research School of Biology, Australian National University, Canberra, ACT, Australia*
[b] *Australian Research Council Centre of Excellence for Coral Reef Studies, Canberra, ACT, Australia*
[c] *Department of Geography, Dawson College, Montréal, Québec, Canada*
[d] *Tropical Water and Aquatic Ecosystem Research, James Cook University, Townsville, QLD, Australia*

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Survival depends on escape responses and when to flee a predator. As a result, factors affecting the escape performance of prey species, including parasite infection, may profoundly influence the outcome of predator–prey encounters. Several hypotheses predict the responses of prey to simulated predator attacks based on intrinsic characteristics such as individual reproductive value and flight costs: as predation risk and reproductive value increase, so should the distance at which an organism begins to flee an escaping predator (flight initiation distance; FID). Conversely, FID should decrease if the costs of fleeing are high. Despite providing testable hypotheses, rarely have these theories been used to predict the escape behaviour of parasitized individuals. The bridled monocle bream, *Scolopsis bilineata*, is parasitized by a large cymothoid isopod, *Anilocra nemipteri*, which attaches above the eye. In this species, ectoparasite infection is associated with increased energy costs and decreased endurance. We investigated the effects of infection on escape performance and FID. Maximum velocity, maximum acceleration, cumulative distance travelled and response latency did not differ between parasitized fish, unparasitized fish and fish that had their parasite experimentally removed. Parasitized fish were smaller, on average, than unparasitized individuals. Smaller, parasitized individuals allowed a threat to approach closer before fleeing (shorter FID) than larger parasitized or uninfected individuals. Since parasite infection has known effects on host growth and metabolism, we suggest that parasitism alters fish escape behaviour as predicted by two nonexclusive hypotheses: (1) by decreasing reproductive value (the asset protection hypothesis) and (2) by increasing the relative costs of fleeing (the economic hypothesis) compared with uninfected and large parasitized fish. The relative importance of each hypothesis in driving the trends observed remains to be tested.

When to escape from a predator is a key behaviour influencing the fitness of mobile species. This decision must consider the time, energy and opportunity costs associated with fleeing as well as intrinsic traits such as size, previous experience and measures of kinematic performance such as maximum achievable speed and acceleration (Domenici, 2010; Januchowski-Hartley, Graham, Feary, Morove, & Cinner, 2011; Lagos et al., 2009; Lima & Dill, 1990; Møller, Grim, Ibanez-Alamo, Marko, & Tryjanowski, 2013; Stankowich & Blumstein, 2005). Extrinsic factors (e.g. predator approach speed, ambient temperature, habitat complexity, distance to shelter) can also influence an individual's decision making when evaluating whether an approaching organism constitutes a threat (Bonenfant & Kramer, 1996; Cooper, 2006; Dill & Houtman, 1989; Domenici, 2010; Domenici, Claireaux, & McKenzie, 2007; Møller et al., 2013; Stankowich & Blumstein, 2005). As a result, escape behaviours involve the complex integration of biotic, abiotic and locomotor variables that should optimize the ratio of benefits to costs of remaining versus fleeing (Cooper & Frederick, 2007, 2010; Domenici, 2010; Lima & Dill, 1990; Stankowich & Blumstein, 2005; Ydenberg & Dill, 1986). Consequently, even slight changes in an organism's ability to react to, evade or outrun a predator can alter individual risk perception and decisions about when to flee an approaching threat.

Parasitic infection can dramatically affect host behaviour and physiology (Barber, Hoare, & Krause, 2000). Several studies have

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* Correspondence: S. A. Binning, Building 116, Daley Road, The Australian National University, Canberra, ACT 0200, Australia.
E-mail address: sandra.binning@mail.mcgill.ca (S. A. Binning).

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linked impaired escape responses (decreased reactivity or locomotor abilities) to parasitism in a range of animals (e.g. Barber, Walker, & Svensson, 2004; Goodman & Johnson, 2011; Libersat & Moore, 2000; Møller, 2008; Perrot-Minnot, Kaldonski, & Cézilly, 2007; Seppälä, Karvonen, & Tellervo Valtonen, 2004). However, these studies generally focus on the effects of endoparasites, many of which have complex life cycles and rely on transmission from prey to predator for their own success (Barber et al., 2000; Poulin, 2010). Consequently, there is a conflict of interest between hosts and parasites regarding predation-relevant behaviours (i.e. parasite increased trophic transmission hypothesis; Barber et al., 2000; Lafferty, 1999; Poulin, 2010, 2013). Conversely, the fitness of directly transmitted parasites, including many externally attached ectoparasites, is enhanced if the host can successfully flee from a predator. As a result, these parasites should be selected to minimize any negative effects on host predation risk (Barber et al., 2000), and hosts should make decisions that will optimize their energy expenditure and chance of escape during a predator encounter. However, ectoparasites may impose additional costs on hosts due to their relatively large size (e.g. Fogelman, Kuris, & Grutter, 2009; Grutter et al., 2011). This may be particularly true in aquatic species given the challenges of moving through relatively dense water (Vogel, 1994). Various studies have found that ectoparasites negatively affect the swimming performance of fish in part by increasing drag, suggesting that the ability to escape an approaching predator may be severally impaired (Grutter et al., 2011; Ostlund-Nilsson, Curis, Nilsson, & Grutter, 2005; Wagner, McKinley, Bjorn, & Finstad, 2003).

The predicted response of parasitized hosts to predator attacks is not necessarily obvious. Flight initiation distance (FID) is the distance at which an organism begins to flee an approaching predator and provides a reliable estimate of an animal's perception of fear or risk (Stankowich & Blumstein, 2005). On the one hand, parasitized fish may be slower than uninfected fish, and thus more vulnerable to predation. One prediction of the economic hypothesis proposed by Ydenberg and Dill (1986) is that FID should increase with higher predation risk. If parasitized fish are less able to outswim a predator once a chase is initiated, we might predict that parasitized individuals will initiate their escape response earlier in an attempt to put more distance between themselves and a predator. On the other hand, the physiological burden imposed by parasites on their hosts means that the energetic and opportunity costs of initiating an escape are much greater relative to unparasitized individuals (Binning, Roche, & Layton, 2013; Godin & Sproul, 1988; Ostlund-Nilsson et al., 2005). The economic hypothesis also predicts that FID should decrease when the costs of fleeing are high (Ydenberg & Dill, 1986). As such, parasitized individuals should only engage in costly flight when a predator approaches close and the threat is high (see Godin & Sproul, 1988; Møller, 2008; Ydenberg & Dill, 1986).

Parasitized or heavily parasitized fish are often smaller than similarly aged uninfected or moderately infected hosts, and previous studies on FID in fishes suggest that size is an important predictor of FID, with small fish generally fleeing at a closer distance to a threat (smaller FID) than larger individuals (Gotanda, Turgeon, & Kramer, 2009; Januchowski-Hartley et al., 2011; Miller et al., 2011). This phenomenon has been largely attributed to the asset protection hypothesis, which predicts that as reproductive value increases, individuals should engage in less risky behaviours in order to protect their reproductive assets (Clark, 1994; Cooper & Frederick, 2007). In fishes, reproductive value typically increases with size (Reinhardt, 2002; Rogers & Sargent, 2001). Thus large fish should increase their FID compared with smaller, less fertile individuals. Consequently, parasites may indirectly decrease host FID through interactions with fish size. Parasites can also directly decrease host reproductive value independent of size by physically castrating hosts (e.g. Fogelman et al., 2009; Lafferty & Kuris, 2009). Based on this logic, we would predict that parasitized individuals should wait longer before fleeing from a threat.

On the Great Barrier Reef, the cymothoid isopod *Anilocra nemipteri* parasitizes the bridled monocle bream, *Scolopsis bilineata*, with up to 30% of fish infected at some sites (Grutter, 1994; Roche, Strong, & Binning, 2013). This species is directly transmitted to its host, although postlarval juveniles (mancae) of some *Anilocra* species may use optional intermediate hosts before settling on a definitive host where they grow into adults (Fogelman & Grutter, 2008). A single isopod typically attaches to a fixed location on one side of the host's head where it breeds repeatedly and can live for several years (Brusca, 1981; Roche, Strong, et al., 2013; Fig. 1). Parasites can grow to 30% of the fish's total length and reduce host growth (Roche, Strong, et al., 2013). *Anilocra nemipteri* does not exhibit any side bias in attachment preference on either the left or right side of the host's body (Roche, Strong, et al., 2013). However, parasitized fish are more highly lateralized (i.e. have a stronger side preference) than unparasitized individuals (Roche, Binning, Strong, Davies, & Jennions, 2013), a behaviour that may enhance escape responses by decreasing reaction time (Dadda, Koolhaas, & Domenici, 2010). These parasites also impair the swimming

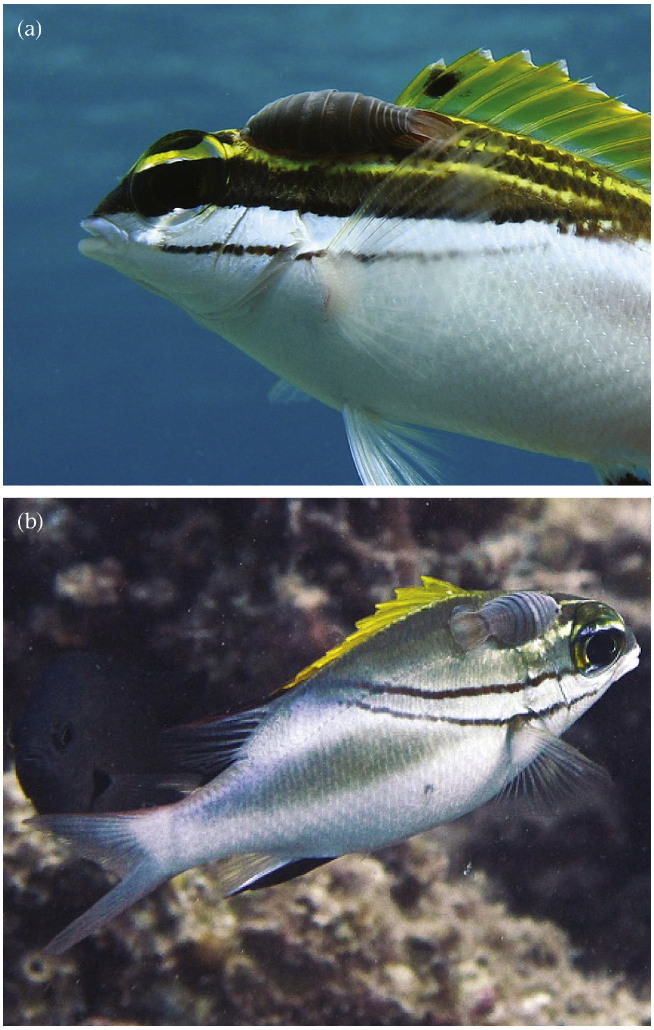

Figure 1. Bridled monocle bream, *Scolopsis bilineata*, with a cymothoid ectoparasite, *Anilocra nemipteri*, attached above the eye. Parasites can attach on either the (a) left or (b) right side of the host. Photo credits: (a) D. Roche and (b) S. Gingins.

ability of *S. bilineata*, mostly by increasing drag at high speeds (Binning et al., 2013). In addition, parasite infection increases energetic maintenance costs and decreases the overall aerobic performance of infected fish (Binning et al., 2013).

We measured the effects of *A. nemipteri* on the fast-start escape performance and risk-taking behaviour of *S. bilineata*. In a laboratory set-up, we filmed escape responses and measured escape performance in fish from three treatment groups: (1) parasitized fish, (2) unparasitized fish and (3) fish with their parasite experimentally removed (test for physiological effects of parasite in the absence of drag). In a field experiment, we then assessed risk-taking behaviour by measuring individual FID elicited by an approaching snorkeller in parasitized and unparasitized fish. Based on escape theory, we predicted that parasitized fish should flee earlier than unparasitized individuals if the presence of the ectoparasite impairs host escape. However, if the parasite does not affect host escape performance, parasitized fish should conserve energy by engaging in more risk-prone behaviour than unparasitized individuals and allow an approaching threat to come closer before fleeing.

METHODS

Study Site and Animal Collection

Parasitized ($N = 28$) and unparasitized ($N = 12$) *S. bilineata* were collected in February and March 2012 using monofilament barrier nets (10 mm stretched mesh) and silicone hand nets from sites around Lizard Island, Northern Great Barrier Reef, Australia (14°40′S, 145°28′E). Fish were transported in aerated 20-litre Handy Pail buckets (maximum four fish per bucket) to the Lizard Island Research Station within 1 h of capture, and transferred with silicone nets to individual holding aquaria (40.0 × 29.0 cm and 18.0 cm high) with a flow-through water system transported directly from the reef. Tanks were kept under a natural light and temperature regime (28 ± 1 °C). All fish were provided with a round PVC shelter for refuge. Fish were fed to satiation once a day with raw prawn and fasted for 24 h prior to the experiments to ensure they were in a standardized (postabsorptive) state that maximizes energy availability for swimming (e.g. Marras, Killen, Claireaux, Domenici, & McKenzie, 2011). Holding tanks were siphoned out daily to maintain high water quality. All animals were kept in aquaria for a minimum of 3 days before performing swim trials to ensure all fish were healthy. Animals were collected and cared for under Marine Parks Permit no. G12/34805.1 issued by the Australian Government Great Barrier Reef Marine Park Authority and the Queensland Government with approval from the Australian National University Animal Experimentation Ethics Committee (permit no.: A2012/02) according to the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes, 7th edition 2007.

Fast-start Experiments

We tested three groups of fish with different infection statuses: unparasitized (mean ± SD fish total body length, BL: 13.2 ± 0.8 cm, $N = 12$), parasitized (11.8 ± 1.1 cm, $N = 16$) and parasite-removed (12.7 ± 1.3 cm, $N = 12$). Parasites were removed using forceps 24 h before the start of the fast-start experiments by holding the fish in a shallow water bath and gently unhooking the isopod (Binning et al., 2013). This procedure took approximately 45 s. Fish resumed normal behaviour within 2 h of being returned to their holding tanks. Experiments were conducted in rectangular acrylic aquaria (70.0 × 60.0 cm and 35.0 cm high) supported by a wooden stand. Water height was maintained at 12 cm for all trials. This height limited movement of the fish in the vertical plane (Langerhans, 2009). A mirror was inclined at a 45° angle below the aquarium to allow filming of the escape responses in three dimensions within the same camera frame. Videos in which vertical movement occurred were excluded from the analyses (<2% of videos). The tank was illuminated by three 150 W spotlights positioned 70 cm above the water. A continuous flow of sea water maintained the water temperature at a constant 28 °C.

Prior to escape response trials, fish were transferred from their holding tanks to the experimental arena using silicone nets to prevent mucus loss and fin damage, and left undisturbed for 30 min to acclimate to the arena. Numerous aerial predators, including raptors and pelagic seabirds, are commonly seen feeding on fishes around Lizard Island. Thus, we used a mechanoacoustic stimulus, which simulates an aerial attack, to induce escape responses in *S. bilineata* (Marras et al., 2011). We attached a 50 ml PVC container filled with lead weights with a string to a platform 30 cm above the water surface. To ensure that the escape response was not initiated prior to the contact of the stimulus with the water, the stimulus fell inside an opaque 15 cm wide PVC tube positioned 1 cm above the water surface (Dadda et al., 2010; Marras et al., 2011). The time of contact of the stimulus with the water surface was clearly visible in the mirror from below. We filmed the responses at 240 Hz with a high-speed digital camera (Exilim EX-FH100, Casio, USA) mounted on a tripod directly facing the aquarium and the mirror. Individuals were stimulated up to three times at 30 min intervals (Marras et al., 2011). The stimulus was dropped when the fish was facing the stimulus at a distance of no more than 10 cm from the bottom of the PVC tube. After the trials, individuals were returned to their holding tanks. In total, we filmed 127 escape responses in 40 fish. Two fish developed a bacterial infection several days following experimentation. These individuals were anaesthetized with an overdose of Aqui-S solution and then euthanized in an ice-slurry. No other individuals showed any signs of sickness, and care was taken to prevent the spread of disease by rinsing nets in a freshwater bath prior to handling different individuals. All healthy fish (38 individuals) were released back to their site of collection within 1 week of trials.

We used the MtrackJ plugin in the ImageJ v. 1.43 software (Meijering, Dzyubachyk, & Smal, 2012) to analyse the escape sequences. We tracked the two-dimensional coordinates of the fish's centre of mass (CoM) every 4.2 ms starting 21 ms (five frames) before and ending 84 ms (20 frames) after the onset of the fish's first movement. The CoM was visually estimated at a proportional distance from the tip of the head corresponding to approximately 29% of an individual's total length. The following escape performance metrics were measured (Domenici & Blake, 1997; Marras et al., 2011): response latency (time between stimulus onset and fish response in ms), size-adjusted cumulative distance travelled (D in fish total body lengths; BL), size-adjusted maximum escape speed (U_{max} in BL/s) and maximum acceleration (A_{max} in m/s^2). Distance–time variables (D, U_{max}, A_{max}) were evaluated within a fixed time period of 58 ms (14 frames), corresponding to the approximate mean duration of stages 1 and 2 of the escape response across all treatments (Dadda et al., 2010; Marras et al., 2011). A five-point moving quadratic polynomial regression (Lanczos, 1956) was used to obtain smoothed values of speed and acceleration, the first and second derivatives of distance (Marras et al., 2011). For each fish, the best value (e.g. highest U_{max} or shortest latency) of each escape performance variable across the three stimulus presentations was chosen for analysis (see Domenici, 2011; Marras et al., 2011).

Flight Initiation Distance

We estimated FID in *S. bilineata* from the lagoon and adjacent reefs in front of the Lizard Island Research Station on calm weather days from July to August 2013. Water depth varied between 2 and 3 m at these sites with a visibility of approximately 15 m. We used snorkellers as the stimulus for flight initiation. Many studies of FID in terrestrial and aquatic systems have used humans as a stimulus to elicit flight (e.g. Bonenfant & Kramer, 1996; Carter, Goldizen, & Heinsohn, 2012; Gotanda et al., 2009; Januchowski-Hartley, Nash, & Lawton, 2012; Lagos et al., 2009; Møller, Nielsen, & Garamszegi, 2008; Perez-Cembranos, Perez-Mellado, & Cooper, 2013). Recent studies found FID estimates to be relatively robust to variation among observers regardless of experience (Guay et al., 2013) or, in aquatic systems, whether observations were made on snorkel or SCUBA (Januchowski-Hartley et al., 2012).

Two snorkellers swam around the reef in search of *S. bilineata*. Only solitary, adult individuals that were foraging or moving slowly over the reef in an open area where they could be approached directly were targeted. Individuals less than 1.5 m from branching corals or other shelter were not approached to avoid the confounding effects of distance to a refuge on FID (see Miller et al., 2011). Similarly, trials were abandoned if individuals began swimming in any direction at a consistent speed before the observer initiated the approach. Once a suitable individual was spotted, we recorded fish infection status (parasitized or unparasitized, hereafter referred to as treatment) and total length (±1 cm; actual error). Before data collection, all observers practised estimating fish length underwater using model fish and objects of various sizes until they reached a precision of ±1 cm. One snorkeller took up a position in direct line of view of the fish at a distance of approximately 5 m. The other snorkeller was positioned off to the side to avoid obstructing the trial. The first snorkeller duck-dived under the water until close to the substrate (within 1 m), and visually relocated the individual. The snorkeller then approached the focal fish at a quick but steady swimming speed, holding two weights marked with flagging tape beside their head, which was assumed to be the onset of the stimulus. When the fish began to flee (i.e. first began to turn away from the approaching snorkeller), the snorkeller dropped one weight where they were, and took a visual landmark of where the fish had been, which was marked with the second weight. The two snorkellers then measured the horizontal distance between the two landmarks with a tape measure to the nearest 1 cm (FID). The observers also scored the strength of an individual's reaction, or flight intensity, on a scale from 0 to 4 as follows: 0: no response (i.e. fish did not move in response to the snorkeller); 1: fish ceased previous activities (i.e. foraging) and moved a short distance away, but did not leave the immediate area; 2: fish changed directions and began moving slowly away from the area; 3: fish changed directions and fled the area at a fast, but constant speed; 4: fish initiated an escape response characterized by a 'C-start' unsteady burst behaviour (Domenici & Blake, 1997). Increasing intensity was assumed to be related to more energetically costly forms of locomotion, and therefore provided an estimate of the costs of flight. Recent studies suggest that *S. bilineata* are strongly site attached and rarely travel far from their small territories during the day (Boaden & Kingsford, 2013, 2012). Therefore, to avoid pseudoreplication, we never sampled two similarly sized fish with the same infection status within 25 m. In total, we measured FID from 104 adult fish ($N = 50$ parasitized, mean BL ± SD = 12 ± 3 cm; $N = 54$ unparasitized, 14 ± 3 cm).

Statistical Analysis

All analyses were performed in R v2.15.2 (R Development Core Team, 2012). Assumptions of the models were assessed with diagnostic plots and Shapiro–Wilks tests for both univariate and multivariate tests. Distance–time variables were normally distributed. Since D and U_{max} are size adjusted (fish BL), and fast-start acceleration is independent of size (see Domenici & Blake, 1997), we used a multivariate analysis of variance (MANOVA using Pillai's trace) to test for an overall difference in distance–time variables (D, U_{max}, A_{max}) between treatments (parasitized, unparasitized and parasite-removed) in the fast-start experiment. Fast-start escape latency violated parametric model assumptions. Therefore we used a nonparametric Kruskal–Wallis rank sum test to assess differences in escape latency between treatments.

Flight initiation distance was square-root transformed to meet model assumptions. As fish body size and treatment were not independent (t test: $t_{101} = -3.97$, $P < 0.001$), we used an analysis of covariance (ANCOVA) with centred fish body size as a covariate to test for the effect of parasitism on FID while controlling for body size (Schielzeth, 2010). Flight intensity did not meet parametric model assumptions. Thus, we used nonparametric Wilcoxon signed-ranks tests to examine the relationship between flight intensity and FID in parasitized and unparasitized fish. We also used nonparametric Spearman correlation to test for a relationship between flight intensity and fish length. Data are deposited in the figshare repository: http//:dx.doi.org/10.6084/m9.figshare.1002130.

RESULTS

Fast-start Experiments

Twelve escape response trials did not elicit a response in fish, and were not included in the analysis (9.4% of trials; three unparasitized, four parasitized, four parasite-removed). Fish infection status (treatment) did not affect overall escape performance in our experiments (MANOVA: $F_{2,72} = 1.79$, $P = 0.11$; Table 1). There was also no difference in escape latency between fish from the three treatment groups (Kruskal–Wallis test: $\chi^2_2 = 3.07$, $P = 0.22$; Table 1).

Flight Initiation Distance

Fish body size estimates from field observations differed between treatments: parasitized fish were significantly smaller than unparasitized fish (t test: $t_{101} = -3.97$, $P < 0.001$, $r = 0.37$). There was an overall effect of treatment and fish body size on FID (ANCOVA: $F_{3,100} = 4.31$, $P = 0.01$, $r^2 = 0.33$). When comparing fish of the same average size, there was a marginally nonsignificant effect of treatment on FID, with parasitized fish tending to have smaller FIDs than unparasitized fish (treatment: $t_{100} = 1.9$, $P = 0.06$; Fig. 2). There was also a marginally nonsignificant interaction between fish size and treatment on FID (interaction: $t_{100} = -1.916$, $P = 0.06$).

There was no relationship between FID and flight intensity for either parasitized (Kruskal–Wallis test: test: $\chi^2_{41} = 44.1$, $P = 0.34$; Fig. 3a) or unparasitized fish (Kruskal–Wallis: $\chi^2_{42} = 49.8$, $P = 0.19$; Fig. 3b). Similarly, there was no difference in flight intensity between parasitized and unparasitized fish (Wilcoxon test: $W = 1283$, $N = 104$, $P = 0.63$, $r = 0.05$). There was no significant correlation

Table 1
Mean ± SEM escape response performance values for unparasitized fish, parasitized fish after removal of their parasite (parasite-removed) and parasitized fish

Treatment	N	Total length (cm)	U_{max} (BL/s)	A_{max} (cm/s^2)	D (BL)	Latency (ms)
Unparasitized	12	13.2±0.2	14.9±1.4	7.0±0.7	0.5±0.1	11.8±2.9
Parasite-removed	12	12.7±0.4	14.1±1.1	6.9±0.5	0.5±0.0	8.3±7.8
Parasitized	16	11.8±0.3	15.8±1.0	7.1±0.4	0.6±0.0	14.6±2.5

Variables measured are fish body lengths (total length, BL; cm), size-adjusted maximum escape speed (U_{max}; BL/s), maximum acceleration (A_{max}; cm/s^2), size-adjusted cumulative distance travelled (D; BL) and escape latency (ms).

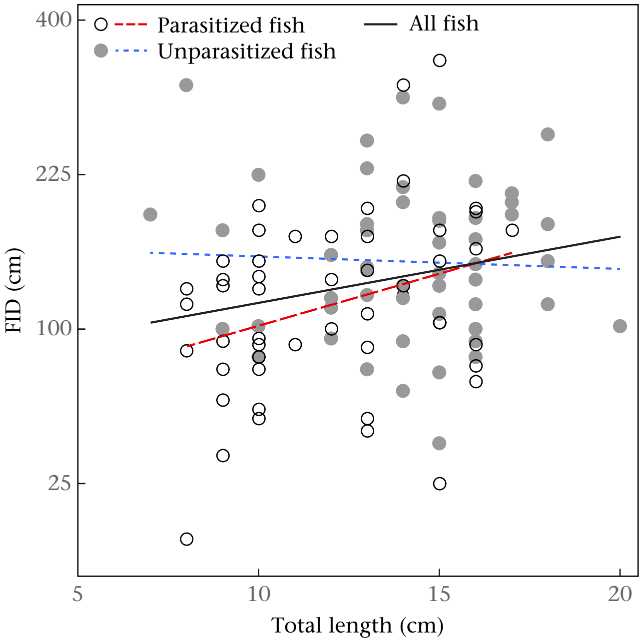

Figure 2. Fish body size (total length; cm) as a function of flight initiation distance (FID; cm) for parasitized and unparasitized fish at Lizard Island. Lines represent the best fit linear regression for parasitized, unparasitized and all fish.

between flight intensity and fish total length (Spearman correlation: $r_S = 0.12$, $N = 104$, $P = 0.224$).

DISCUSSION

Escape behaviour is fundamental to the survival of mobile organisms when faced with a predator. Despite recent studies reporting decreased sustained swimming ability in parasitized fish (e.g. Binning et al., 2013; Grutter et al., 2011; Ostlund-Nilsson et al., 2005), we found that infection by large cymothoid ectoparasites did not impair the escape performance of *S. bilineata* in our experimental trials. During a simulated attack, parasitized fish were as likely to perform a characteristic fast-start response, and responded as quickly, as fast and travelled as far from the stimulus as healthy, unparasitized individuals. Similarly, when we removed the ectoparasite from infected individuals, performance did not differ from that of healthy fish. Binning et al. (2013) found that parasite removal restored overall prolonged swimming performance and aerobic capacity in *S. bilineata*, suggesting that the physiological effects of the parasite on its host are rapidly overcome. Our results support this conclusion, but also suggest that large ectoparasites have no discernible impact on individual escape performance metrics. Fast-starts are anaerobically powered, rapid accelerations followed by a change in direction, and tend to be mediated by large Mauthner-cell neurons in the brain (Domenici, Blagburn, & Bacon, 2011). This stereotyped reaction has implications for individual performance. Both locomotor and nonlocomotor performance in fast-start behaviours tend to be repeatable through time, suggesting that escape performance is an intrinsic characteristic of an individual (Marras et al., 2011). Our results suggest that parasitic infection does not alter this intrinsic characteristic during a single attack, perhaps because single bursts are short behaviours that even sick fish can engage in effectively. When accelerating from rest, most energy is used to counteract the forces of inertia rather than drag, which is more important during prolonged swimming. Inertial forces should be similar for both parasitized and unparasitized individuals during burst behaviours, which may partly explain why no differences in maximum performance were found between treatments. However, recovery from energetically costly burst activity depends on a fish's overall aerobic capacity, or aerobic scope. Parasitized *S. bilineata* have a smaller aerobic scope than unparasitized individuals (Binning et al., 2013), which suggests that repeated escapes or the ability to evade a predator during a sustained chase may be greatly impaired even if an individual's maximum kinematic performance abilities are not.

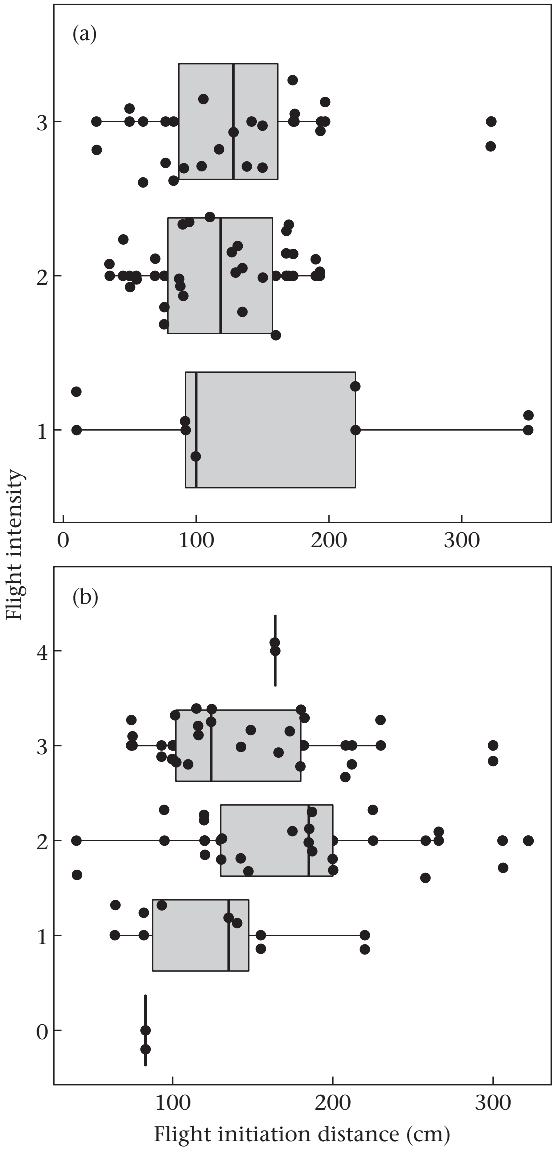

Figure 3. Inverted box plots showing the relationship between flight initiation distance and flight intensity for (a) parasitized and (b) unparasitized fish. Individual flight initiation distance (FID; cm; closed circles) is plotted on top of the boxes to illustrate the distribution of the data. The box plots show the median and 25th and 75th percentiles; the whiskers indicate the values within 1.5 times the interquartile range.

Parasitized fish, being more highly lateralized, should respond faster to a threat than unparasitized individuals (Roche, Binning, et al., 2013). However, we did not find any advantage imparted by ectoparasites on escape latency. Our mechanical stimulus simulated an aerial attack by an avian predator. As *A. nemipteri* attaches above the eye (Fig. 1), it may decrease the visual range or acuity of its host especially in response to an aerial stimulus. As a result, a strong side bias may still elicit a more rapid response in parasitized individuals when facing a sudden attack in the same horizontal plane. The relative importance of lateralization in improving performance during aerial versus aquatic attacks remains to be tested.

The effect of parasite infection on escape behaviour in the field was highly influenced by fish size. Overall, we found that large fish fled at a greater distance than small individuals, although this trend was much less apparent for unparasitized than parasitized fish (marginally nonsignificant interaction; see Fig. 2). This result is generally consistent with previous studies on a range of coral reef fishes, which have also found increases in FID with fish total length (Gotanda et al., 2009; Januchowski-Hartley et al., 2011; Miller et al., 2011). The asset protection hypothesis predicts that individuals with higher reproductive value should be more risk adverse, and therefore increase their FID (Clark, 1994; Cooper & Frederick, 2007). In fishes, fecundity, and thus reproductive value, increases dramatically with size (Reinhardt, 2002; Rogers & Sargent, 2001). Thus, we would expect to see greater risk-averse behaviour (higher FID) in larger individuals. Host growth is often adversely affected by parasite infection. The isopod *Anilocra apogonae* infects the cardinalfish *Cheilodipterus quinquelineatus*, and effectively castrates its host by reducing gonad size, body length and weight compared with similarly aged uninfected fish (Fogelman et al., 2009). At sites around Lizard Island, parasitized *S. bilineata* are on average 25% smaller than unparasitized individuals, suggesting that *A. nemipteri* also stunts fish growth and reduces the reproductive value of its host (Roche, Strong, et al., 2013). As a result, parasite infection may influence FID partially through its effect on fish size.

We predicted that, if there is no cost of parasitism on escape latency or distance-derived kinematic traits, parasitized fish should wait until a predator has approached closer before fleeing. This prediction was based on the economic hypothesis and the assumed higher costs of flight in infected versus uninfected fish: as costs increase, FID should decrease (Ydenberg & Dill, 1986). For an average fish body size, there was a marginally nonsignificant effect of parasite infection on FID suggesting that the direct effect of parasites on FID is weak. However, the effects of parasitism on FID were particularly apparent for small fish, and less so for large fish. Small parasitized fish took more risks and fled at shorter FID than larger parasitized fish, which behaved more similarly to risk-averse uninfected fish of all sizes (Fig. 2). Metabolic costs are higher when fish are infected with ectoparasites (Binning et al., 2013; Grutter et al., 2011; Ostlund-Nilsson et al., 2005). This suggests that the observed relationships among size, parasite infection and FID might also be explained by flight and opportunity costs, which may be particularly important for small, parasitized individuals. As a result, parasite infection may alter risk aversion behaviour in smaller parasitized individuals in two complementary ways: (1) by decreasing reproductive value and (2) by increasing the relative costs of fleeing compared with larger parasitized and/or unparasitized fish. Once flight was initiated, the intensity of the response was similar regardless of size or infection status. We quantified flight intensity as the strength of the reaction, with higher intensities presumed to be more energetically costly than lower intensity reactions. As a result, it appears that fish trade off the timing of the response rather than the intensity as a way of modulating their escape behaviour. By reacting to only the most threatening stimuli, the risk-prone behaviour of small, infected fish is likely to be an energy-saving mechanism in the long run.

Although we have interpreted our results based on predictions derived from evolutionary theory, nonadaptive explanations for the patterns are also possible. Parasites may obstruct fish vision, and reduced FID may simply be a consequence of a decreased detection distance in smaller, infected fish. Visual acuity is also expected to increase with eye size, and thus fish size (McGill & Mittelbach, 2006). Therefore, it is possible that larger fish are able to detect an approaching predator and react earlier than smaller individuals. Isopods may also reduce host condition (e.g. Adlard & Lester, 1994; Fogelman et al., 2009), which may cause individuals to behave in nonadaptive ways.

Parasitism, by definition, imposes a cost on hosts. Consequently, infected individuals must develop strategies that minimize these costs while ensuring their own survival. We found that kinematic escape performance was unaffected by ectoparasite infection. However, small, parasitized *S. bilineata* behave differently from larger parasitized fish and unparasitized conspecifics when facing a threat in the field. Small, infected fish engage in riskier behaviour, probably as a result of their increased costs of fleeing and/or lower reproductive value compared with larger fish. This risky behaviour could be adaptive for both hosts and parasites by reducing the energetic and opportunity costs of flight except in the most threatening of circumstances.

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