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REPORT

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Efficacy of passive integrated transponder tags to determine spawning-site visitations by a tropical fish

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Abstract Individual identification of organisms is a crucial part of assessing the processes that influence small-scale distribution patterns and the maintenance of social organizations. This study evaluates the use of passive integrated transponder (PIT) tags to quantify small-scale space use in a marine damselfish, *Pomacentrus amboinensis* (Pomacentridae). Implanting small PIT tags in the body cavity of fish down to 5.2 g with a hypodermic needle had no adverse effect on mortality or growth over a 47-day tank study. A 63-day field study also showed that tags had no influence on body condition as measured by Fulton's condition factor (K), gonadosomatic indices, or plasma cortisol levels (a physiological indicator of stress). The utility of PIT-tag technology is illustrated by preliminary information from a study on the diel periodicity of visitations to male nest sites by female *P. amboinensis*. Information suggests that there is a strong periodicity in visitations. Females first entered the nest site at 03:20 h, with 88% of the total of 4 h 45 min that females spent within a single nest occurring prior to dawn. Despite this, 81% of the total visitations to the nest occurred after dawn, with most being very brief, averaging 17 s. No one female monopolized access to the nest, with nine females accounting for 90% of the time that females spent within the nest site. This methodology will be particularly useful in the study of activity rhythms, patch dynamics, and social interactions in a wide range of marine organisms.

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Introduction

Behavioral studies often require the recognition of individuals. Studies of space use by fishes in marine environments have used a variety of methods to enable individual identification during in-situ behavioral observations. Typically, external tags have allowed identity to be determined without the need for further capture. Techniques are varied and include the use of acrylic paint, latex or fluorescent elastomer tattoos (e.g., Thresher and Gronell 1978; Malone et al. 1999), fin clipping (e.g., Sale 1971), heat branding (e.g., Jones 1987), colored beads (e.g., Bean et al. 2002), and the presence of parasites (e.g., Buckley and Blankenship 1990). However, obtaining information on space use is prohibitively labor intensive, and many questions can only be addressed through the use of some form of automated identification system.

In the marine environment, various methods are available for the remote assessment of spatial movement, which are appropriate for different magnitudes of spatial resolution. Satellite tags have been used on mammals, marlin, and sharks to examine local- (100's m) to geographic- (10's to 1,000's km) scale movement patterns (e.g., Stevens et al. 1998; Ferrero et al. 2000; Graves et al. 2002). Ultrasonic implant tags, which use either remote beacons or handheld and boat-mounted detection units, are useful for local- (Zeller and Russ 1998; Zeller 2002) to regional- (1,000 s m; Zeller 1999) scale movements. Passive integrated transponder (PIT) tags are useful for recording the transit of individuals past specifically placed sensory gateways (e.g., salmonids exiting or entering river systems, McCutcheon et al. 1994; Achord et al. 2003) and for smaller, within-site movements (below 10 s m; e.g., Burns et al. 1997; Roussel et al. 2000). It is at this spatial scale that most variability in abundances occurs for shallow marine fish species (Jones 1988; McCormick 1995), since this is the level at which organisms interact and at which most site-attached species make behavioral choices (e.g., Öhman et al. 1998).

PIT tags have been available for over a decade, and have been used extensively to determine the effectiveness of salmonid hatcheries and the stream fidelity of various stocks (e.g., Prentice 1990). The use of PIT tags in the marine system has been restricted to the individual identification of various organisms (fishes, Quartararo and Bell 1992; urchins, Kalvass et al. 1998; octopus, Anderson 1997) in tag-release programs to determine demographic parameters, such as mortality (e.g., Achord et al. 2003). Because of their low weight (0.06 g), relatively small size, unlimited functional life, and billions of individual codes, PIT tags are ideal for tagging small fishes. The present study evaluates the use of PIT tags to assess visitations of tropical marine fish to a spawning site. We explore the assumption that the presence of a PIT tag in the body cavity of an individual does not adversely affect its physiology, growth, reproductive biology, and survival. This is particularly important due to the relatively large size of the tag compared to the space available within the body cavity. The utility of the system is illustrated using data collected on female visitation frequencies during the spawning season of a tropical damselfish, *Pomacentrus amboinensis* (Pomacentridae). This is the first automated use of the technology to track resource use in a marine species.

Material and methods

Study species and site

The study was conducted at Lizard Island (14°38′S, 145°28′E) on the northern Great Barrier Reef, Australia, during December 2000 to February 2001 (laboratory study), and December 2002 to February 2003 (field study). A field study was undertaken at a shallow site (4 m at high tide) on the reef edge within Blue Lagoon. The model species was *P. amboinensis*, a protogynous hermaphrodite that is common on coral reefs of the Indo-Pacific. Like many damselfishes, males guard a demersal nest site (in this case, an upturned clam shell or plate coral) during the austral summer (October to the following March, depending on latitude). Females are attracted from the neighboring reef to spawn on the male's nest site. Little is known of the social system, which was an impetus for the present study. At the study site, females become mature at approximately 45-mm standard length (SL; McCormick, unpublished data).

PIT-tag technology

PIT tags are composed of an electromagnetic coil, tuning capacitor, and microchip sealed in a tubular glass enclosure (Fig. 1a). The chip is programmed with a unique identification code that is transmitted to the reading system when the transponder is activated by a low-frequency radio signal from the antenna. The reader system used in the present study is a commercially available table-top loop antenna attached to a waterproof data-logger. These were originally designed to detect tagged salmonids diverted through 6-inch plastic pipes as they return to their natal river to spawn. The antenna and data-logger (Destron FS2001FT, Fig. 1b) were each enclosed in underwater housings made of 20-mm-thick Perspex, and linked together by a 6-m twisted-pair cable. The antenna and data-logger were enclosed in separate housings to enable the data-logger, together with its batteries, to be brought to the surface without disruption to the male nest site, of which the antenna housing was an integral part. Housings were pressure tested to 50-m water depth. Cables longer than 6 m were not found to reliably detect tags entering the antenna field once the antenna was immersed in seawater (20, 12, and 8 m were also assessed). Three 12-V rechargeable gel-cell batteries that yielded enough power for 40-h continuous use were used to power the reader. We chose the smallest available PIT tag for use in this project (11.5 mm long, 2.1 mm in diameter; Destron-Fearing TX1400BE, ISO 134.2 kHz; Fig. 1a), due to the small size of the fish we wished to tag—39 to 62 mm SL (5–11 g). We estimate that the tag takes up to 10–15% of the overall space in the body cavity.

Tank study

A laboratory study was undertaken to determine whether the insertion of a PIT tag into the body cavity of adult *P. amboinensis* affected mortality or growth. Thirty-five adult *P. amboinensis* of a broad size range (40–65 mm SL) were collected using anesthetic clove oil (Munday and Wilson 1997) and a fence net from the back-reef of Lizard Island, and randomly allocated to two laboratory treatments—PIT-tagged, and non-PIT-tagged. Immediately upon being brought back to the laboratory, the fish were placed one at a time into a small clip-seal plastic bag containing aerated seawater to prevent scale damage during manipulation. All fish were measured with calipers (±0.1 mm), and individually marked with a fluorescent elastomer tattoo (Northwest Industries Inc.) to allow individual identification. These tattoos were undertaken with a 29-gauge hypodermic needle through the clip-seal bag into the musculature, avoiding the head and body cavity region. A PIT tag was then inserted into the body cavity of 20 fish of a size range, using a 12-gauge hypodermic needle attached to a specially adapted syringe, which ejected the PIT tag upon depression of the syringe plunger. Care was taken to insert the needle about 5 mm anterior and slightly lateral of the anus.

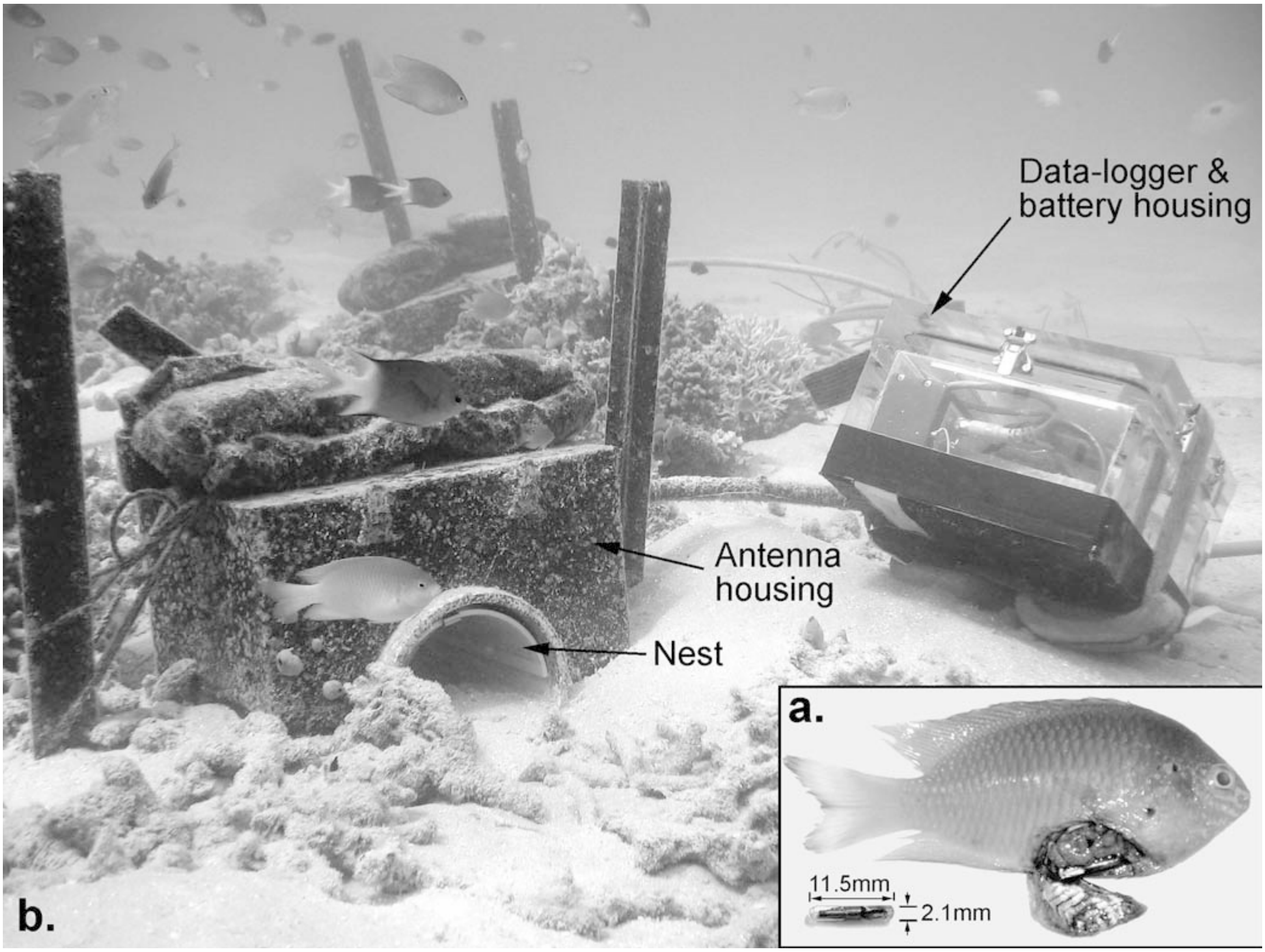


Fig. 1a Dissected *P. amboinensis* showing position of the PIT tag and its dimensions (65-mm standard length male). **b** A male guarding a nest site surrounded by a Perspex housing containing the radio antenna anchored by two vertical starpickets, linked by a 6-m cable to a second housing containing the data-logger and batteries

The needle was slipped under the scales at the penetration site, and the tip of the needle was used to make an incision just large enough for the PIT tag to be inserted, with minimal penetration of the needle to avoid damage to the alimentary tract. Each PIT tag was sterilized in an iodine-based antiseptic solution prior to use. Fish were randomly placed into two 1,000-l outdoor tanks containing many shelter sites, with 10 PIT-tagged and 7–8 non-PIT-tagged fish in each. Fish were fed daily with chopped pilchards, and re-measured and scanned for PIT tags after 47 days.

Field test of influence of PIT tags on physiological condition

To determine the influence of PIT tags on the physiological condition of females, 12 PIT-tagged females and 12 non-tagged females from an adjacent section of reef were collected by fence net and clove oil, 63 days after the initial implanting of tags, as part of a field study (see below). Once caught, fish were placed into a plastic bag, and 150–200 μl of blood was immediately sampled underwater by caudal puncture, using a 300-μl syringe with a 27-gauge hypodermic needle. The blood was stored on ice until returned to the laboratory where it was centrifuged and the plasma frozen for later cortisol determination. Fish were killed by cold shock and returned to the laboratory for processing. Standard length was measured with callipers, and total body and ovary weight were recorded. Four measures of condition were compared between tagged and non-tagged groups, using analysis of variance (ANOVA): standard length (mm); Fulton's condition factor (K, i.e., weight (kg)/SL(m)^3); gonadosomatic index (GSI = ovary weight (g)×100/body weight (g)); and plasma levels of the stress-related hormone, cortisol (ng/ml; Pankhurst and Van der Kraak 1997). The ANOVA assumptions of homogeneity of variance and normality were examined using residual analysis.

Plasma cortisol was assayed according to the radioimmunoassay (RIA) protocol of Pankhurst and Carragher (1992). Fifty microliters of plasma was extracted with 500 μl ethyl acetate by vigorous agitation for 10 min, followed by centrifugation at 10^4 *g* for 3 min. Duplicate 15-μl aliquots were evaporated in assay tubes and resuspended in 200 μl phosgel buffer. The radioactive tracer used for the RIA was (1,2,6,7-^{3}H) cortisol (TRK407; Amersham, UK), while the antiserum was developed against cortisol-3-carboxymethyloxime-bovine serum albumin raised in rabbits (UCBA907/R1Y; Accurate Chemical and Scientific Corporation, NY). This antiserum is reported to have cross-reactivities to corticosterone (1.8%) and aldosterone (<0.02%). Cortisol within the samples was quantified in a single assay.

Example of field application

To determine the utility of PIT tags to assess the periodicity of visitations to male nest sites by female *P. amboinensis*, PIT-tag readers were set up on two adjacent nest sites. Four weeks prior to installation of readers, 20 natural nests guarded by male *P. amboinensis* were replaced with artificial nesting surfaces consisting of half of an 18-cm-diameter PVC water pipe (30 cm long), split lengthwise and roughened. These pipes presented a uniform, concave nesting surface of similar dimension and defensibility as natural nests. Nests were monitored for eggs daily for 2 weeks and, based on this information, two adjacent, reproductively successful males were chosen as the recipients of PIT-tag readers. All potentially reproductive females within an approximately 12-m radius of the nest sites were captured with a fence net and clove oil, transferred into a plastic bag and tattooed, and PIT tagged in situ following the procedure used for the laboratory experiment. Females were positively identified after capture by the morphology of the genital aperture: males have small conical papillae, while females have a tubular opening. Forty females between 39 and 62 mm SL were tagged. After tagging, fish were transferred to a 10-l plastic bag for 10 min prior to release back at their site of capture. Upon release, fish showed few signs of stress, did not hide, and often resumed feeding within 1 min of release. Two weeks after females had been tagged, PIT-tag readers were established at the male nest sites. Establishment entailed displacing the male nest site 1 m to one side and, as carefully as possible, digging a 15-cm-deep hole in the sand and coral rubble to allow the antenna housing to be half buried. Two 6-inch vertical starpickets were used to hold the positively buoyant housing in position (Fig. 1b). The male nest site was then placed close to its original position, next to the entrance of the antenna housing. Over the next day, the nest was moved further into the entrance of the antenna housing, until the male associated the housing with its nest site. The housing for the datalogger and batteries was placed on sand 3 m further from the main reef. This second housing was brought to the surface at low tide every 30–40 h to allow batteries to be changed and data to be downloaded to a laptop computer. Nests were monitored for 58 days. The reader was set on a 3-s delay, so that readings from the antenna (determined up to every 60 ms) were stored only every 3 s. Examination of the time that the antenna field was disrupted, together with the tag identity, allowed us to determine approximately how long an individual stayed within the nest site, and how this changed with time of day and size of fish. To demonstrate the utility of the PIT-tag system, data from the visitations from only one of the two PIT-tag readers are presented.

Results and discussion

The fundamental requirement of using a tagging technique on a study organism for an ecological investigation is that tagging does not detrimentally affect the organism's demographic parameters (mortality, growth, fecundity, and associated energy allocation), or its behavior. PIT tags were shown to be an efficient way of tagging a small marine fish, with minimal disruption and no short-term detectable adverse effects on their life-processes.

PIT tags were effectively used on *P. amboinensis* down to 5.2-g body wet weight. It took 3–5 min to capture, sex, measure, and tag *P. amboinensis* underwater. With the exception of one PIT-tagged fish that jumped out of its tank, there was no tag-induced mortality during the 47-day laboratory study. There was also no rejection of tags in any of the 19 remaining fish, and the tag site healed over within 2–4 days. This supports a similar finding for captive Eurasian perch, *Perca fluviatilis*, where fish were successfully tagged down to 1.7 g (55-mm fork length; Baras et al. 2000). In contrast to the present study, Baras et al. (2000) found that injection of the tag into the body cavity induced 40% mortality within the first 2 weeks after tagging. Likewise, PIT-tag-induced mortality in the Nile tilapia, *Oreochromis niloticus*, was found to be high and size-related, with small fish (<3 g) sustaining 90% mortality in the first day, while fish of 7–15 g suffered 50% mortality (Baras et al. 1999). This study also found that of the three tagging techniques investigated (injection, incision, and incision with a suture), fish receiving the syringe-implanted tags sustained by far the highest mortality. Combined with the present study, evidence suggests that the reaction to the tagging procedure and the likelihood of inducing significant mortality may be species-specific. Tagging protocols should be tested for each target species prior to their large-scale use.

In the present study, fish that received a PIT tag had the same growth trajectory as that of non-PIT-tagged fish over the 47-day duration of the experiment (Fig. 2). This suggests that despite the tag taking up a significant amount of space in the gut cavity, the tag did not reduce

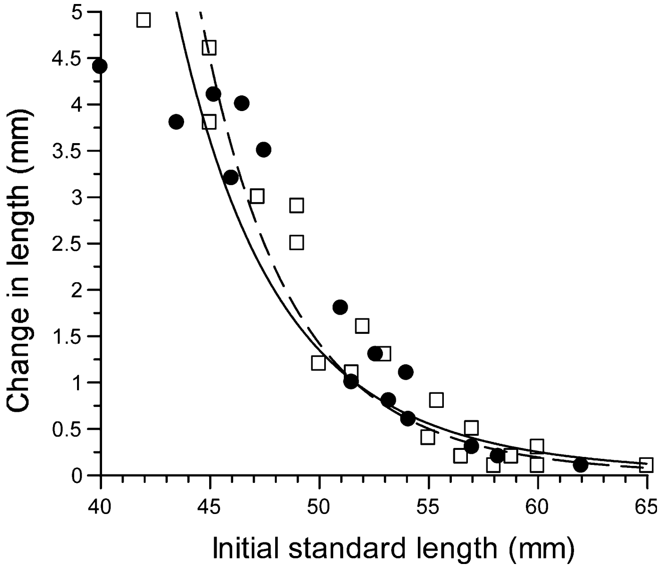


Fig. 2 Comparison of growth between *P. amboinensis* that had been implanted with a PIT tag (*squares*) and control fish (*circles*). Power curves are fitted to the data

food intake to the extent of reducing growth rates. Few studies have quantified the effect of internal tags on growth, concentrating rather on mortality. Those studies that measured growth have found that internal tags (PIT or small acoustic transmitters) have had no effect on the growth of juvenile fish (salmonids; e.g., Moore et al. 1990; Peterson et al. 1994).

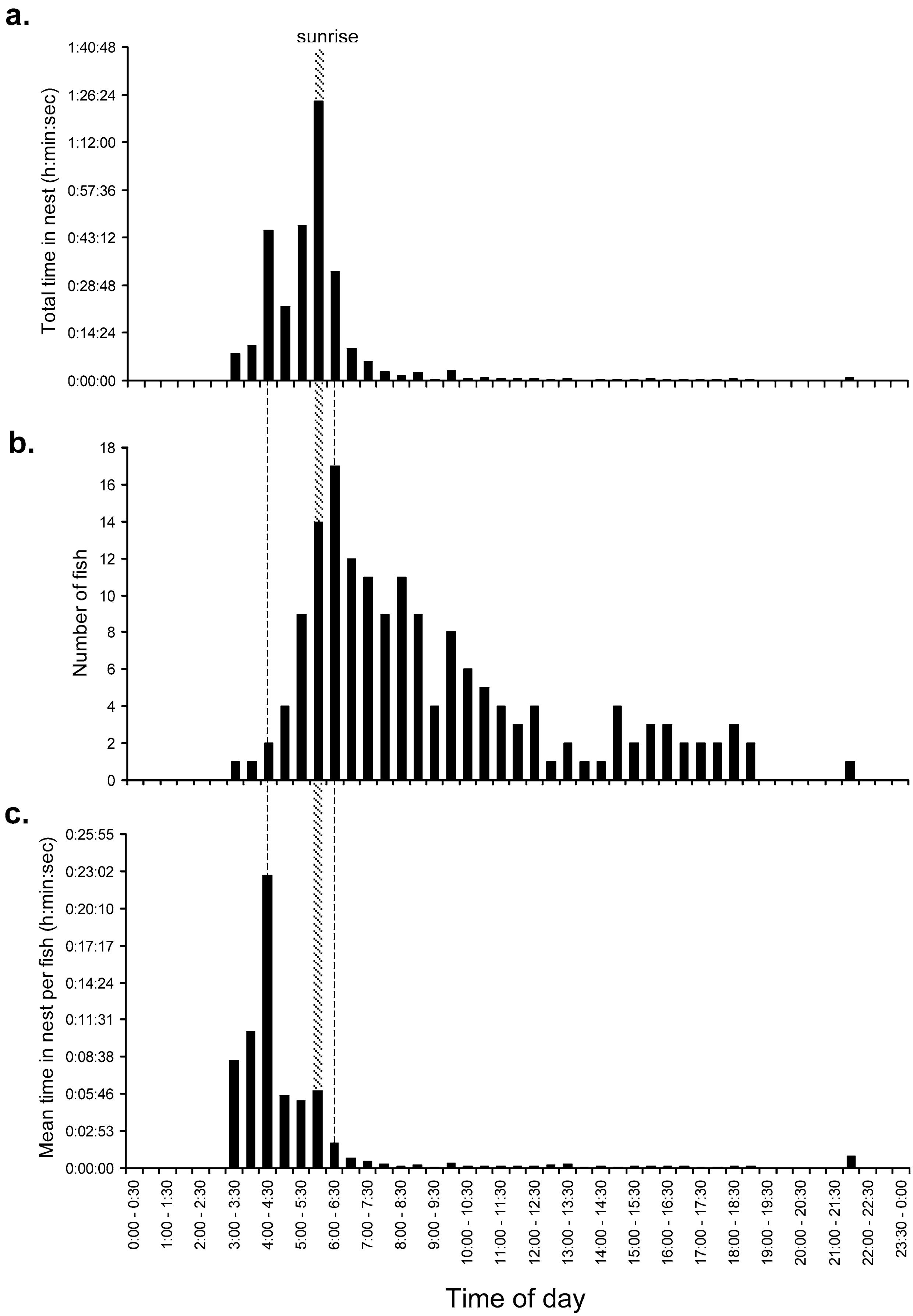
PIT tags had little impact on the long-term physiological condition of the fish. There was no difference in the Fulton's K (or bulk) between tagged and non-tagged fish (Table 1). Moreover, levels of the stress-associated hormone, cortisol, in the blood plasma were considerably lower in the control (non-tagged) fish, although this was not significantly different at 0.05 α level. Interestingly, the gonadosomatic index was significantly lower in the control females compared to those fish that had PIT tags taking up space in their body cavity (Table 1). This higher reproductive condition and lower stress of the PIT-tagged females may be associated with the non-random choice of male nests on which the PIT-tag readers were established. Male nests were chosen that displayed the highest reproductive success over a previous 2-week monitoring period. Since the number of egg clutches a male receives is likely to be limited by the number of reproductive females in the vicinity, females in the vicinity of less successful males may be expected to have a lower gonadosomatic index. Together, this evidence suggests that the insertion of PIT tags into the body cavity of *P. amboinensis* females had no adverse effects on their physiological condition.

Table 1 Comparison of mean traits of female *P. amboinensis*, PIT-tagged and left in their natural environment for 63 days, with that of undisturbed control fishes. Degrees of freedom (Df) of the hypothesis and the error terms, significance values ($p < 0.05$ in italics) for the test of equality of means (one-way ANOVA), and treatment means (standard errors) are given

Variable	Df	*p*-value	Mean PIT-tagged	Mean control
SL	1.20	0.493	54.49 (1.41)	53.03 (1.54)
Fulton's K	1.20	0.161	45.73 (0.98)	43.62 (1.07)
GSI	1.17	*0.008*	3.49 (0.51)	0.90 (0.66)
Plasma cortisol	1.19	0.066	40.55 (10.33)	71.40 (11.93)

Fig. 3a–c Diel cycle in visitations by female *P. amboinensis* to a nest site during the 9-day period when the male was guarding eggs. **a** Total time that females were in the nest; **b** number of females within each 30-min time interval entering the nest; and **c** amount of time spent within the nest (per fish). Sunrise (*shaded*) occurred between 05:46 and 06:00 h

The identification of females with PIT tags allowed us to examine their visitations to a male nest site in a detail not previously possible. Previous studies have quantified the seasonal and daily patterns of spawning activity by recording the presence or absence of new egg clutches within a nest (e.g., Goulet 1995). While this has provided useful information, no information is available on the daily timing of nest-site activities, because the collection of such data is prohibitively time-consuming by non-automated means. In the present study, the deployment of PIT-tag readers on the male nest sites enabled the collection of detailed information on the frequency and duration of nest visitations by individually identifiable females. Females were within the nest for a total of 4 h 45 min during the 9-day period (of the 63 days monitored) when eggs were present in the nest. Visits to the nest occurred throughout the day, with the exception of the time interval 22:00–03:00 h (Fig. 3). Visitations by females started at 03:20 h, with 88% of the time spent within the nest occurring prior to dawn. Whether most of the spawning occurs prior to dawn also, as has been suggested (Meekan 1992), is currently unknown. More females entered the nest in the 30 min following dawn than at any other time (17 females), but each fish spent on average only 1 min 56 s within the nest (Fig. 3). Females spent the longest time within the nest between 04:00 and 04:30 h, with two females spending 24 and 21 min, respectively. While the majority of the time females spent within the nest occurred prior to dawn, 81% of the visitations occurred after dawn. These visits were generally very brief, averaging 17 s. Over this 9-day period, the nest was visited by 24 different females. No one female monopolized access to the nest, with nine females accounting for 90% of the time that females spent within the nest site (Fig. 4); the other 15 females accounted for the remaining 10%. This evidence suggests that *P. amboinensis* has a polygamous mating system, typical of many damselfishes (Thresher 1984). The high number of short visits to the nest by females, especially after dawn, suggests that females may be assessing the number of the eggs within the nest, and the ability of


a.
sunrise
Total time in nest (h:min:sec)
1:40:48
1:26:24
1:12:00
0:57:36
0:43:12
0:28:48
0:14:24
0:00:00
b.
Number of fish
18
16
14
12
10
8
6
4
2
0
c.
Mean time in nest per fish (h:min:sec)
0:25:55
0:23:02
0:20:10
0:17:17
0:14:24
0:11:31
0:08:38
0:05:46
0:02:53
0:00:00
0:00 - 0:30
1:00 - 1:30
2:00 - 2:30
3:00 - 3:30
4:00 - 4:30
5:00 - 5:30
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17:00 - 17:30
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23:30 - 0:00
Time of day

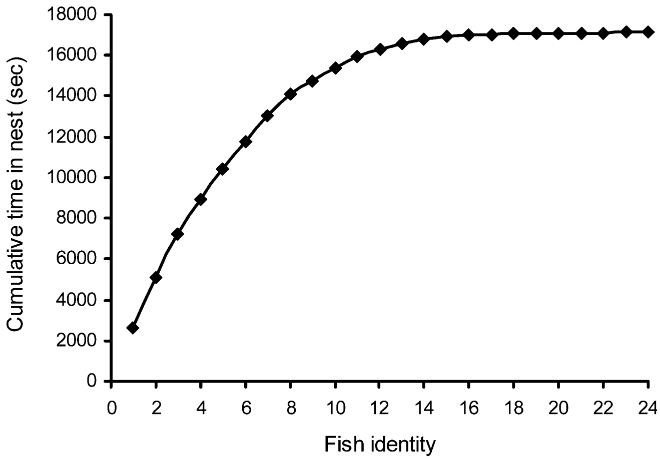

Fig. 4 Cumulative total time spent within one male *P. amboinensis* nest site by 24 females out of a total 4.6 h from a 9-day period when the male was guarding eggs

males to guard and maintain those eggs. A number of studies have shown females to assess male success prior to future spawning, with males who have a history of attracting spawning females, and successfully guarding clutches, receiving more eggs than less successful males (Gronell 1989; Kraak and Groothius 1994; Forsgren et al. 1996).

This study demonstrates the effectiveness of PIT-tag technology in quantifying visits to a specific site for a marine species in an unprecedented detail. Installation in the field, with minimal disruption to the nest-guarding male or associated females, allowed us to obtain the first realistic estimates of nest-site visitation by females for a coral reef fish. The recent development of a flat-plate PIT-tag detector (Nunnallee et al. 1998) could increase the utility of this technology and the range of questions addressed. Linking this technology with readily available and inexpensive infrared cameras will enable us to obtain information on marine animal behavior on a detail not previously possible. This methodology could be easily adapted to many other types of marine vertebrates and large invertebrates, and will be particularly useful in the study of activity rhythms, patch dynamics, and social interactions.

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