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# Photographic identification based on unique, polymorphic colour patterns: A novel method for tracking a marine crustacean

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**Abstract**

Mark–recapture techniques are an important tool for estimating population parameters of vagile organisms. However, the application of marks (tags) to crustaceans is problematic due to tag-loss during moulting of the exoskeleton. Accordingly, we investigated the use of external colour patterns to distinguish (via photographic identification) individuals of a common marine crustacean (painted crayfish, *Panulirus versicolor*). Colour patterns were found to be highly polymorphic and individually unique, such that all crayfish in a sample of 59 could be individually identified. When 30 of these crayfish were recaptured after 6–36 months at liberty, colour patterns were unchanged, despite moulting during the inter-census period. It was concluded that (1) photographic identification is an effective method for tracking *P. versicolor* through time and space, and (2) this method of identification may be useful in capture–recapture investigations of other invertebrate species that display polymorphic colour patterns. This result is significant given the logistical, ecological and ethical problems of attaching tags to crustaceans, as well as invertebrates in general.


*Keywords:* Crustacea; Intra-specific variation; Mark–recapture; Photographic identification; Polymorphism; Spiny lobster

## 1. Introduction

Mark–recapture techniques have been used extensively in studies of animal ecology, most often for determining patterns in abundance, growth, survivorship and behaviour of vagile organisms (Stonehouse, 1978). However, it is not always possible to mark (tag) certain types of animals, either because they are problematic to tag, or they are protected by law from disturbance (e.g. cetaceans, Hammond et al., 1990). As an alternative, it is often possible to identify individual animals from variations in natural marks and (or) polymorphic colour patterns. If so, identification may be achieved by comparing photographs (of individual animals) that were taken at different points in time. Although photographic identification (photo-id) has been widely employed to study vertebrates (e.g. Persat, 1982; Hammond et al., 1990; Doody, 1995; Anderson and Goldman, 1996; Kelly, 2001), this technique has apparently never been used to track the movements of invertebrates, despite the presence of polymorphic colour patterns in many species (e.g. Vianna, 1986; Whiteley et al., 1997).

One group of invertebrate animals that is particularly difficult to track through time and space is the Crustacea

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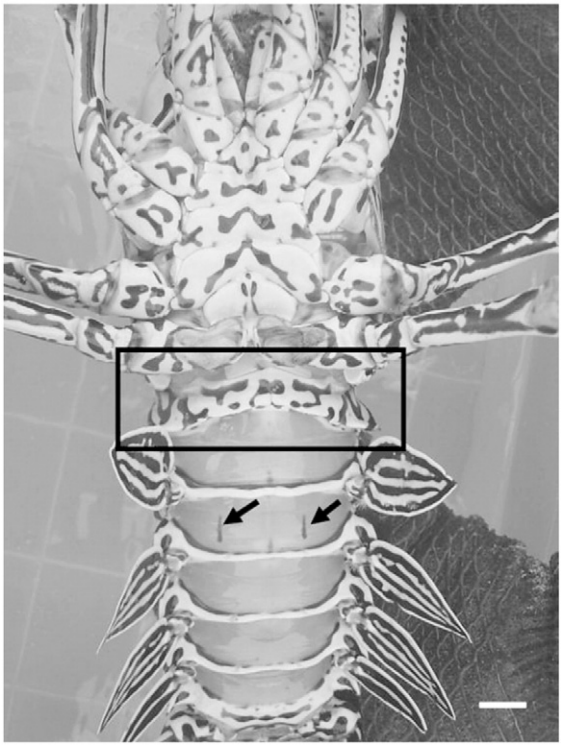

Fig. 1. Photograph showing the location of the heavily calcified region of the first abdominal sclerite in *Panulirus versicolor*. The inserted rectangle defines the portion of each photograph that was excised for reliability analysis. Arrows show the location of secondary marks (i.e. internal elastomer tags) that were used to confirm individual identity. Scale bar = 20 mm.

(Phylum Arthropoda), because any tag or mark that is attached to the exoskeleton is lost during ecdysis (moulting). Attempts to overcome this problem have resulted in the development of tags that are either sub-skeletally anchored in the musculature or completely embedded in it (Scarrat and Elson, 1965; Weingartner, 1982; Uglem et al., 1996). However, invasive tags can alter the growth, behaviour and (or) survivorship of host animals by causing injury, disease, physical hindrance or physiological interference (Scarrat, 1970; Bergman et al., 1992; Linnane and Mercer, 1998; Courtney et al., 2001). The perfect tag for crustaceans, therefore, is one that is both non-invasive and resistant to ecdysis. Unfortunately, such a tag does not presently exist.

Given the complexity of colour patterns in some species of crustaceans (Jones and Morgan, 1994), it may be possible to identify individual animals by photo-id, thereby avoiding the problems associated with tagging this group of animals. In species with strongly calcified exoskeletons (e.g. spiny lobsters), colouration results from the deposition of pigments in the pro-cuticle layer (Ghidalia, 1985). However, even if individuals can be distinguished from one another at one point in time, colour patterns may not be consistent over long-term periods, since the pro-cuticle is replaced each time the animal moults (Ghidalia, 1985). Proper evaluation of photo-id in capture-recapture studies of crustaceans must therefore include an assessment of the stability of colour patterns across the moult cycle.

The painted crayfish *Panulirus versicolor* (Latreille) is a Palinurid (spiny) lobster that is distributed throughout coral-reefs of the Indian and Pacific Oceans (Munro, 2000). This species has striking colour patterns, thus making it an ideal candidate in which to evaluate the effectiveness of photo-id for tracking crustaceans. The specific objectives of this study were to (1) evaluate the use of polymorphic colour patterns for identifying individuals of *P. versicolor*, and (2) assess the stability of these colour patterns in *P. versicolor* over a long-term period (36 months).

## 2. Materials and methods

### 2.1. Capture and recapture of crayfish

The study was conducted at Northwest Island (23° 18′ S, 152° 43′ E) in the southern section of the Great Barrier Reef, Australia. The coral-reef surrounding Northwest Island is typical of this region, with modest numbers of *P. versicolor* inhabiting protective coral shelters (dens) at various depths across the reef. Individuals of *P. versicolor*

Table 1
The number of *Panulirus versicolor* captured and recaptured during each of six censuses conducted at Northwest Island, Great Barrier Reef, Australia

| Capture date | Total number captured [a] | Recapture date | Number recaptured [b] | Time at liberty (months) |
|---|---|---|---|---|
| Dec. 2003 | 7 | June 2004 | 2 | 6 |
| June 2004 | 23 | Dec. 2004 | 2 | 6 |
| Dec. 2004 | 14 | June 2005 | 4 | 6 |
| June 2005 | 17 | Dec. 2005 | 2 | 6 |
| Dec. 2003 | 7 | Dec. 2004 | 1 | 12 |
| June 2004 | 23 | June 2005 | 3 | 12 |
| Dec. 2004 | 14 | Dec. 2005 | 3 | 12 |
| Dec. 2005 | 19 | Dec. 2006 | 7 | 12 |
| Dec. 2003 | 7 | June 2005 | 4 | 18 |
| June 2004 | 23 | Dec. 2005 | 4 | 18 |
| June 2005 | 17 | Dec. 2006 | 6 | 18 |
| Dec. 2003 | 7 | Dec. 2005 | 2 | 24 |
| Dec. 2004 | 14 | Dec. 2006 | 2 | 24 |
| June 2004 | 23 | Dec. 2006 | 7 | 30 |
| Dec. 2003 | 7 | Dec. 2006 | 1 | 36 |
| Dec. 2006 | 21 | – | – | – |

[a] Includes recaptured individuals.
[b] Values sharing the same 'capture date' are not mutually exclusive (some individuals were recaptured more than once).

show considerable site fidelity (Frisch, 2007a), thus making this species ideal for a capture–recapture study.

Crayfish were captured by hand (with the aid of SCUBA) and restrained in a shallow, water-filled box onboard a nearby support vessel. Each crayfish was then photographed, tagged and measured (see below). This process took approximately 5 min, after which each crayfish was manually returned to its den. The location of all dens was recorded by global positioning system (GPS) to aid subsequent recapture.

The study site was censused over ten consecutive days on six occasions — December 2003, June 2004, December 2004, June 2005, December 2005 and December 2006. 'Initial captures' were obtained during censuses 1–5, while 'recaptures' were obtained during censuses 2–6. All recaptured crayfish were processed (i.e. photographed and measured) and released again, thus making it possible for multiple recaptures (and multiple photographs) of the same individual at intervals of 6 months or more. In each case, time-at-liberty was dependent upon the date of initial capture.

A secondary mark was used to confirm the identity of individual animals. This consisted of a small elastomer tag (Northwest Marine Technology, Shaw Island, WA, U.S.A.) that was inserted into the abdominal musculature (Uglem et al., 1996). These tags were considered unlikely to alter the colour patterns of crayfish. Tags were applied using the longitudinal method described by Woods and James (2003), with individual identification facilitated by using different combinations of tag colours and tag positions.

Carapace length ($L_c$; i.e. between the supra-orbital ridge and the posterior, dorsal edge of the carapace) was determined using vernier calipers and the occurrence of moulting inferred from growth increments accrued during the inter-census period (Pitcher, 1993).

### 2.2. Photographic identification and reliability tests

A waterproof digital camera (Sony DSC-P9, Tokyo, Japan) was used to photograph the ventral side of each crayfish. Digital images were downloaded to a computer

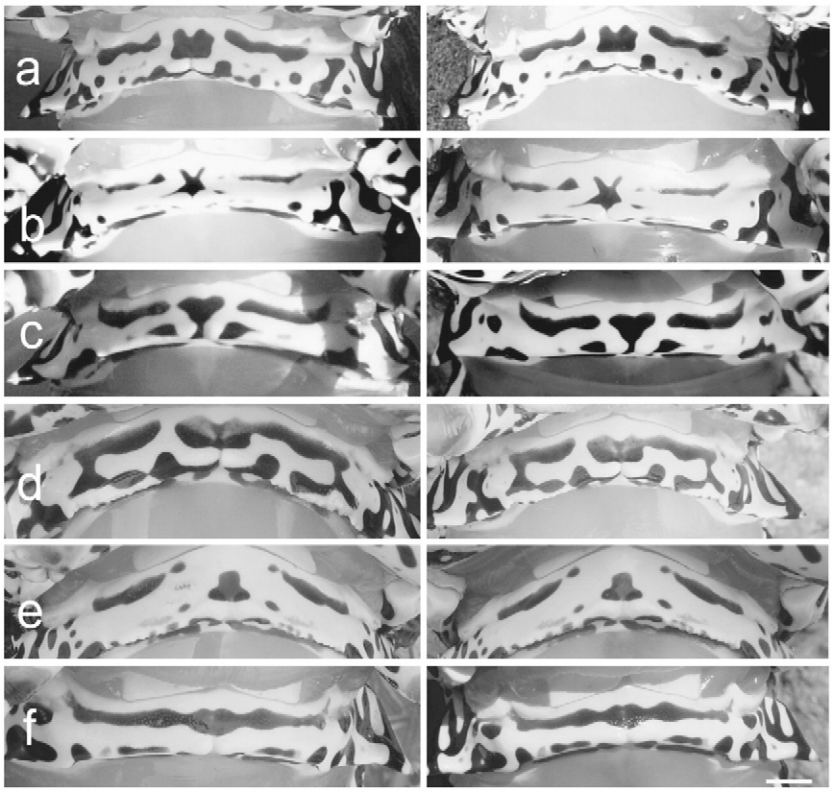


Fig. 2. Photographs illustrating unique, polymorphic colour patterns in six individuals of *Panulirus versicolor* (a, b, c, d, e and f). The left photograph was taken after initial capture, while the right photograph was taken upon recapture. The time at liberty (i.e. time between photographs) for these individuals was (a) 6, (b) 12, (c) 18, (d) 24, (e) 30 and (f) 36 months. All six individuals moulted during their time at liberty (inferred from changes in $L_c$). Scale bar = 10 mm.

and viewed using Photostudio software (ArcSoft, Fremont, U.S.A.). Due to the complexity of colour patterns in *P. versicolor*, only the heavily calcified region of the first abdominal sclerite was used for comparative analyses (Fig. 1). This small section of exoskeleton was chosen because it was (1) easy to define in photographs, (2) always void of spermatophores (tar-spots), eggs and epiphytes, and (3) rarely affected by injury.

One photograph was selected to represent each capture and recapture event per crayfish. Next, each photograph was trimmed to remove secondary information, and the remaining portion printed (without enlargement) on high resolution paper. These (trimmed) photographs were assigned a label (depicting the census during which each photograph was taken) and presented to three independent judges who were asked to identify individual crayfish (i.e. crayfish that were captured during two or more censuses) based on colour patterns. Errors in identification were recorded as 'false' if an incorrect match was made, or 'missed' if a correct match went unnoticed.

## 3. Results

### 3.1. Capture and recapture of crayfish

A total of 56 *P. versicolor* were tagged and photographed between December 2003 and December 2005 (Table 1). This comprised 28 males ($L_c$ 43–148 mm) and 28 females ($L_c$ 94–156 mm). Thirty individuals were recaptured after 6, 12, 18, 24, 30 and (or) 36 months, giving an overall recapture rate of 54%. A further three (previously uncaptured) crayfish were photographed during the final census in December 2006.

Ninety percent (27/30) of recaptured crayfish moulted during the inter-census period (inferred from changes in $L_c$). Absolute growth for most crayfish was in the range of 5–22 mm $L_c$, although one small crayfish (initial $L_c$=43 mm) grew by 37 mm $L_c$.

### 3.2. Photographic identification and reliability tests

Colour patterns on the first abdominal sclerite were found to be highly polymorphic, with noticeable intra-specific differences in the size, number, shape and arrangement of pigmented areas (Fig. 2). All 59 crayfish that were examined could be distinguished on the basis of these characteristics. Colour patterns were also stable over time, irrespective of whether ecdysis had occurred (Fig. 2). All noticeable changes in colour pattern were very subtle and did not interfere with individual identification.

Two judges rapidly identified 29 of the 30 recaptured crayfish (some of which were recaptured more than once), with the remaining judge able to identify all of the recaptured crayfish. When the first two judges were shown their 'missed' identifications, both agreed that the relevant photographs were obviously of the same individual. There were no 'false' identifications by any of the judges.

## 4. Discussion

Two assumptions of photo-id in capture–recapture studies are that (1) colour patterns are distinctive enough to ensure that all individuals in a population can be identified with a high degree of accuracy, and (2) colour patterns are permanent enough to allow all individuals to be identified through time (Pennycuick, 1978). In relation to the first assumption, it is clear that colour patterns in *P. versicolor* are highly polymorphic and unique to individuals, at least for the 59 animals examined. Furthermore, these animals were distinguished relatively easily (even though only a small section of exoskeleton was considered), thus suggesting that photo-id could be used to identify many, perhaps thousands of different animals. One explanation for the degree of polymorphism in *P. versicolor* is that external colour patterns function in intra-specific recognition, as has been suggested for other crustacean species (Dunham, 1978; Vannini and Gherardi, 1981; Hall-Spencer et al., 1999).

With respect to the second assumption, it is evident that colour patterns in *P. versicolor* are stable over time and resistant through ecdysis. Growth increments in adults of this species are commonly 2–3 mm $L_c$ (Frisch, 2007b), thus suggesting that most recaptured crayfish moulted 2–7 times during the inter-census period. The case of the small individual ($L_c$=43 mm at capture and 80 mm at recapture) indicates that terminal colour patterns are attained well before the establishment of reproductive maturity (65–82 mm $L_c$; George and Morgan, 1979; MacDonald, 1982). These results are consistent with the notion that colour patterns are both genetically determined (Ghidalia, 1985) and permanent (at least in sub-adult and adult crayfish).

The use of photo-id in capture–recapture studies can incur two types of errors: false matches and missed matches (Pennycuick, 1978). Considering the degree of polymorphism in *P. versicolor*, errors of the former type were unlikely to have been encountered. In contrast, errors of the latter type were probable, since the judges were inexperienced, and each was required to perform over 4000 manual comparisons. Furthermore, some matches were less obvious than others, not because of indistinct colour patterns, but because the photographs varied in quality (e.g. angle and focus). The probability of missed matches could therefore be substantially reduced

by (1) training of personnel in techniques of identification, (2) using computer programs to match photographs (e.g. Arzoumanian et al., 2005), and (3) acquiring higher quality photographs. This would ensure that all crayfish in the population have an equal chance of being correctly identified as a 'recaptured' animal.

The primary advantage of using photo-id in capture–recapture studies is that it circumvents the need for tags, thereby avoiding the logistical, ecological and ethical problems of attaching an artificial object to a wild animal. However, using photo-id also has disadvantages. Firstly, it removes the potential for tag reporting by the public, since there is no tag to return. Secondly, it is labour intensive, especially when large numbers (i.e. thousands) of animals are involved. Although the handling time of each crayfish in this study was approximately 5 min, this could be substantially reduced in future studies by obtaining photographs directly at the site of capture (i.e. underwater).

In summary, this study has demonstrated that exoskeletal colour patterns in *P. versicolor* are highly polymorphic, individually unique, resistant to ecdysis, and stable through time. It is concluded, therefore, that photo-id is an effective method for tracking *P. versicolor* over long-term periods. It is anticipated that photo-id will also be useful for tracking other vagile invertebrates that display individual variation in colour patterns, such as molluscs (e.g. Whiteley et al., 1997) and other crustaceans (e.g. Vianna, 1986).

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