JOURNAL OF CRUSTACEAN BIOLOGY, 27(3): 393–398, 2007

INDIVIDUAL IDENTIFICATION OF DECAPOD CRUSTACEANS I: COLOR PATTERNS IN ROCK SHRIMP (*RHYNCHOCINETES TYPUS*)

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ABSTRACT

We examined whether color patterns of a marine crustacean may serve to distinguish between individuals and to successfully identify individuals after one molt. Digital images of the rock shrimp *Rhynchocinetes typus* were digitally processed in order to obtain their diffraction pattern by means of Fast Fourier Transformation (FFT). All diffraction patterns were correlated with a phase-only filter using images of rock shrimp before and after one molt. To determine the degree of similarity of color patterns among rock shrimps, correlation of diffraction pattern was performed. This approach showed that among shrimps the color pattern of the cephalothorax is mainly homogenous (~83%) in both distribution and intensity. However, the observed degree of variability (~17%) was sufficient to distinguish between individuals. Furthermore the general color pattern of each individual persisted after one molt and all 14 individuals could be recognized based on their color pattern. It is concluded that this non-intrusive method for distinguishing among shrimp individuals may be a useful tool that could be developed and used in the future instead of expensive and intrusive tagging techniques.

INTRODUCTION

In many studies on the ecology or culture of crustaceans, the accurate identification of individuals is imperative. This need is easily resolved in short-term experiments because labels can be attached to the exoskeleton allowing individual identification. However, in long-term experiments, this method is not feasible because marks attached to the exoskeleton are lost during molting.

In general, mark-recapture studies that extend over long time periods (several months to many years) have used two principal approaches: 1) artificial labels attached to an organism, e.g., bird-rings or fin-tags, or 2) natural marks, e.g., fin shapes, scars or other morphological finger-prints (for overview see McGregor and Peake, 1998). These techniques can also be classified into categories of intrusive methods where animals must be manipulated at least once, and non-intrusive methods where manipulation is not necessary or can be reduced to a minimum. Tags attached to the body or injected into internal tissues may increase mortality or affect the behavior of organisms (Smith et al., 2001). In contrast, natural body marks may change over time, in particular when studies extend over very long time periods.

In crustaceans, diverse techniques of artificial tagging have been employed in the past in order to permit identification of individuals over long time periods. These include coded discs or colored synthetic monofilaments attached to the exoskeleton (Caceci et al., 1999). Moreover, microchip technology and visible implant fluorescent elastomers have also been employed as an invasive tagging technique (Schmalbach et al., 1994; Jerry et al., 2001; Arce et al., 2003; Woods and Jones, 2003). These methods involve the insertion of labels into different body parts, and therefore the main advantage is that the mark may be retained through molting. In addition, there exist other mechanisms that produce marks on the body of the animals, such as tattoos, and freeze- or heat-branding (Fletcher et al., 1989; Schmalbach et al., 1994). Molecular methods (fingerprinting) have also been used as markers but to our knowledge these have not yet been applied in mark-recapture studies (McGregor and Peake, 1998; Jerry et al., 2004).

While natural body marks or morphological patterns have been widely used to identify and recognize individuals in other organisms (see McGregor and Peake, 1998), these have been rarely considered for crustacean studies. This is surprising since many crustacean species exhibit diverse patterns of coloration and spination. For example, many shrimp species are known for their conspicuous color patterns, such as for example *Hymenocera picta* (Wickler, 1973), *Periclimenes rathbunae* (Spotte et al., 1991), or *Rhynchocinetes striatus* (Nomura and Hayashi, 1992). For *H. picta*, Seibt and Wickler (1972) had remarked that individuals can be distinguished based on the distinct color patches. Also, in other shrimp species individuals can be recognized individually using color patterns, e.g., in *Sclerocrangon borealis* (B. Sainte-Marie, personal communication). Recently, Detto et al. (2006) demonstrated that individual female fiddler crabs in some species can be distinguished based on color patterns on their carapace and that males may use these marks to recognize female neighbors. If individual variability of color patterns is common in crustaceans, this may not only have evolutionary consequences but could also be employed by crustacean researchers to distinguish between individuals. In addition to particular color patterns, many crustacean species feature diverse patterns of spines on their body, such as for example arcturid isopods, lithodid crabs, or spiny lobsters. MacDiarmid et al. (2005) confirmed that the pattern of coloration and spines in the rock lobster *Jasus edwardsii* can be used to identify individuals, employing these marks or patterns as a fingerprint. The use of natural characteristics can have two important advantages: 1) marks may persist through time, and 2) the stress of manipulation during application of artificial tags is reduced. However, besides the study by MacDiarmid et al. (2005) to our knowledge it is not known for crustaceans whether natural body marks persist throughout several molts. It is thus important to examine the temporal stability of natural body marks on individuals in order to evaluate the usefulness of this method.

Technological advances may aid in using natural marks for individual identification. During recent years methods based on image analysis have been increasingly used for the identification of patterns in organisms or objects. Techniques of digital identification of shapes have long been used in marine ecology, for example in plankton research (Pech-Pacheco et al., 2001a, b). In most of these methods a focal image (of an object of known identity) is compared to a sample image to reveal the identity of the sample. In order to be able to compare two images these are transformed from the spatial plane to a frequency distribution of different color intensities (= diffraction pattern). All fields in the image are assigned an intensity of color and then transformed into the diffraction pattern of the image using the Fast-Fourier transformation. The resulting diffraction pattern of the focal image can then be correlated with that of the sample image (for details of the method see Pech-Pacheco et al., 2003). Up to present, these techniques have primarily been used to identify species and not to distinguish between individuals of the same species. Nevertheless, individual identification via natural body marks has a long-standing tradition in human society and also in ecological studies (Markowitz et al., 2003).

In this study we examine the usefulness of natural color patterns of a crustacean to distinguish and track individuals over time. We used the caridean shrimp *Rhynchocinetes typus* as a study organism, because this species is characterized by conspicuous color patterns composed of three principal colors (creamish-white, yellow, dark-red). We took digital images of the animals and employed a comparative analysis of images by correlation of diffraction patterns (CDP). The main objectives of this study were: 1) to determine the degree of individual variability of color patterns for *R. typus* by means of CDP, and 2) to examine whether the individual-specific color pattern persists after a molting event.

Materials and Methods

Collection and Maintenance of Rock Shrimp

Shrimp were collected on the pier of Universidad Católica del Norte located in La Herradura Bay, Coquimbo, Chile (29°59′S, 71°23′W). The shrimp were captured using baited traps, and transferred to a communal tank with flowing seawater, air supply, and ad libitum food (dead fish, crushed mollusks, and ascidian colonies with their epibionts). The shrimp were acclimated in these conditions over a period of four days, and then sexed according to Correa et al. (2000). A total of 14 males of similar size with morphometric characteristics corresponding to the ontogenetic state "typus" were chosen for the experiments (for details see Correa et al., 2000).

Each of the 14 shrimp was maintained in a small container with flowing seawater with a small amount of food. The shrimp were maintained for 2 months until each individual had molted at least once. Measurements of all shrimp were taken at the start of the experiment and after the first molt: total body length from the first rostral spine to the tip of the telson, length of the right maxilliped, length of the cephalothorax, width and length of the right chela.

Image Analysis and Correlation of Diffraction Patterns (CDP)

The degree of variability in the coloration pattern among individuals and the possibility of recognizing individuals after the first molt were evaluated by means of correlation of diffraction patterns (CDP). Images of the whole shrimp were taken at the start of the experiment (pre-molt) and after one molt (post-molt) with a digital camera Kodak model DX6340, and with a focal distance of 40 cm. Figure 1A illustrates the main steps of image processing. Step I shows the captured image (2272 × 1704 pixels) from each shrimp. In step II from each individual image a subimage of 100 × 100 pixels was obtained between the anterior region and the posterior border of the cephalothorax, because this area is the most regular in shape in order to capture standardized images, i.e., avoiding focus distortion due to flexed body parts such as e.g., appendices. In step III, the Fourier transformation was applied to the subimage in order to obtain frequency data (diffraction pattern). Then in step IV, from the diffraction pattern a subimage phase-only filter (S_{POF}) was generated according to Horner and Gianino (1984). For each focal individual (of known identity) a S_{POF} was produced and compared to all other individuals (here considered as sample images) via correlation analysis (steps II', III' and IV' respectively). The correlation was generated according to the convolution theorem between the focal and sample images, step V, using the software MATLAB 6.0 (Copyright 1984-2000, The MathWorks, Inc.). First, an autocorrelation for the S_{POF} of each individual was calculated, and this was then compared to the above inter-individual correlation values. The autocorrelation value was set as 100% and the percentage of the highest peak of the inter-individual correlation was calculated.

In order to reveal the average variability in color patterns between individuals, the pre-molt S_{POF} of each individual (based on images taken before the molt) was compared against that of all other 13 individuals. The resulting correlation values for all 14 individuals were then compared using a one-way ANOVA. In this analysis, the individual was the factor with n = 14 levels (the shrimp individuals 1 to 14). The dependent variable was the correlation coefficient value to all other individuals (n = 13 observations per level). The assumptions of ANOVA, homogeneity of variances and normality were verified by means of Bartlett and Kolmogorov-Smirnov tests, respectively (Zar, 1999). The overall degree of variability in the color pattern was estimated by the mean percentage value of all combined inter-individual correlations.

To examine the persistence of color patterns over a molt cycle, the initial pre-molt S_{POF} of each individual was compared to its post-molt S_{POF}. The correlation value between the same shrimp, before and after the molt was denominated as intra-individual correlation. Likewise, correlation values between different shrimps were denominated inter-individual correlation. The overall degree of variability in the color pattern was estimated by the mean percentage values of all combined inter-molt correlations against the autocorrelation values for each individual before the molt.

Results

Individual Identification of Rock Shrimp by Correlation of Diffraction Patterns (CDP)

The general process of digital correlation is illustrated in Fig. 1B. Comparison of two identical images via the CDP process produced a maximum correlation peak denominated autocorrelation (I vs. II). Likewise, comparison of the same shrimp before and after the molt revealed a positive and central correlation peak in the correlation plane (I vs. III). In contrast, comparing the images of two different individuals showed no strong correlation peak (I vs. IV), whereas in the comparison between two different individuals before and after the molt no central correlation peak was observed (negative correlation).

Variability of Coloration Pattern in Rock Shrimp

Statistical analysis showed significant differences (ANOVA $F(13) = 2.62$, $P = 0.016$) in the intensity of correlation

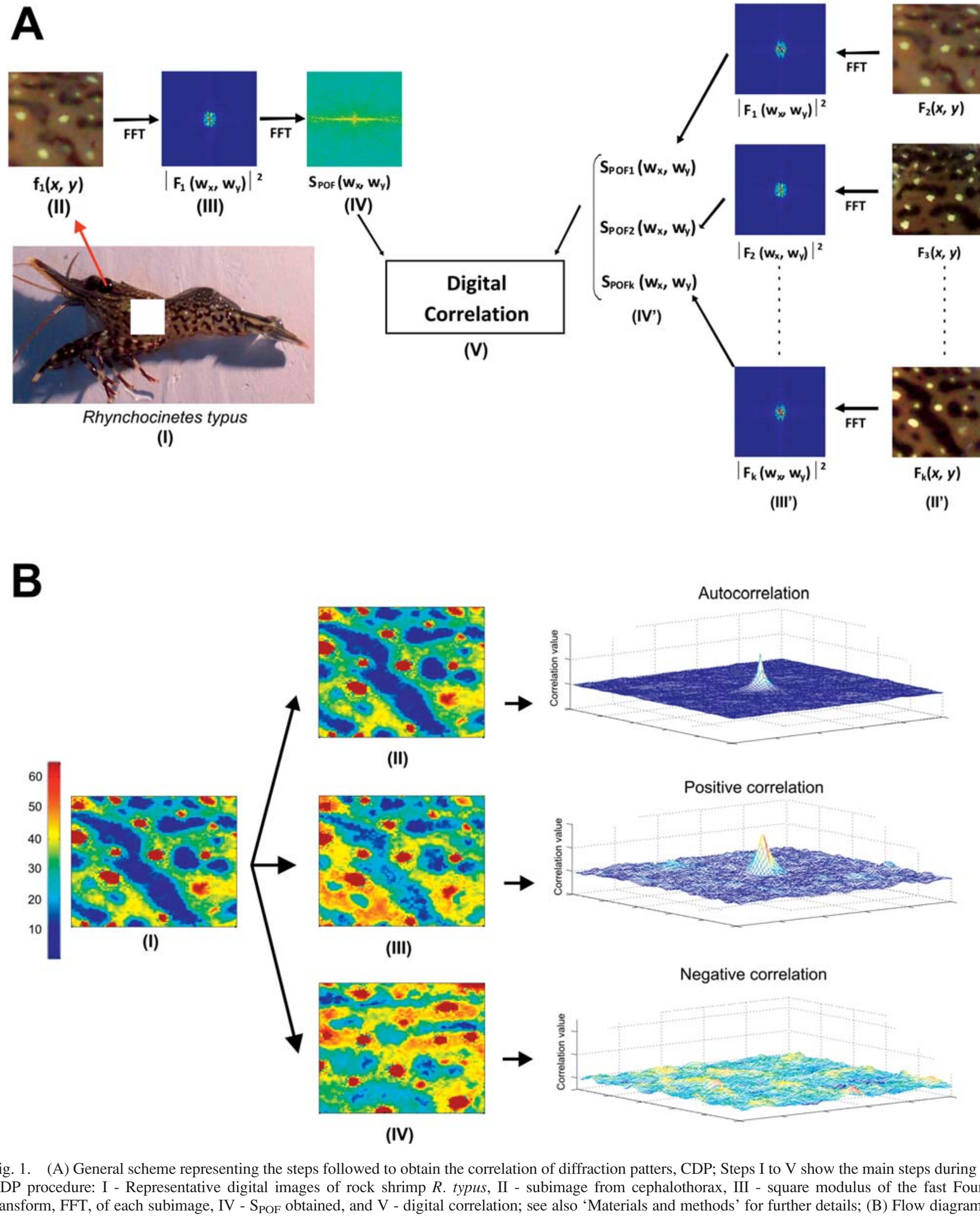

Fig. 1. (A) General scheme representing the steps followed to obtain the correlation of diffraction patters, CDP; Steps I to V show the main steps during the CDP procedure: I - Representative digital images of rock shrimp *R. typus*, II - subimage from cephalothorax, III - square modulus of the fast Fourier transform, FFT, of each subimage, IV - S_{POF} obtained, and V - digital correlation; see also 'Materials and methods' for further details; (B) Flow diagram of digital identification by correlation of diffraction patterns, CDP; Images I to IV show: I - Image of focal individual, II - copy of image I, III - sample image of focal individual after one molt, and IV - sample image of a shrimp different from the focal shrimp.

between color patterns among the 14 studied shrimp individuals. The variability in the coloration pattern was estimated by the degree of similarity from the inter-individual correlations. The relatively low correlation level observed among the pre-molt shrimp is mainly due to the fact that only a minor fraction of the color pattern varied statistically among the tested individuals (16.9 %). Thus, the coloration pattern of rock shrimp is relatively homogeneous

Table 1. Values of inter-individual percentage correlation of male rock shrimp *R. typus* at the start of the experiment; the pre-molt S_{POF} of each individual is compared with the pre-molt S_{POF} of all other 13 individuals (see 'Materials and methods' for details).

Ind	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		18.7	16.3	19.2	18.6	21.6	24.6	14.3	25.1	24.2	17.6	20.8	25.8	16.1
2			14.0	12.1	12.0	15.2	15.3	12.1	10.2	15.1	10.0	15.3	17.5	13.1
3				15.0	12.3	15.0	26.4	17.1	19.9	21.7	16.0	14.6	17.3	19.0
4					14.6	26.0	18.5	11.3	12.8	18.9	13.2	16.3	17.4	15.8
5						21.5	27.4	10.9	28.1	24.5	10.6	19.2	30.1	12.9
6							18.4	13.0	14.6	15.6	13.1	12.8	22.4	13.9
7								7.8	17.3	14.4	9.6	10.4	10.8	18.0
8									24.5	25.3	16.9	24.3	19.0	15.0
9										23.8	10.9	13.3	21.3	14.3
10											10.0	13.7	21.6	20.7
11												16.8	16.6	14.5
12													7.7	7.4
13														13.8
14														

(83.1 %). Nevertheless, the percentage of variation based on coloration pattern is sufficiently high to distinguish among the limited number of individual rock shrimps used herein (see Table 1).

Intra and Inter-Individual Correlation after One Molt

The process of intra-individual identification was carried out by CDP between two images of the same shrimp, before and after the molt. Table 2 shows the correlation values for all combinations among pre- and post-molt shrimps. For all 14 examined shrimp, the highest correlation values were found for the intra-individual comparisons (compare within rows). Thus, maximum correlation values allowed the positive identification of all post-molt shrimp (Table 2), i.e., a correct match between the pre- and the post-molt image of each individual is obtained. Interestingly, the shrimp with the lowest correlation values (individual #10) also showed a comparatively high correlation to other shrimp before molting (see Table 1).

Discussion

We tested a new non-intrusive method of individual identification based on natural body marks (herein color patterns). Identification of individuals is of great importance both in applied as well as in basic research on crustaceans (Smith et al., 2001). Digital systems based on natural characters are continuously improved and are increasingly used to monitor the presence and quantity of organisms in their natural environment. The introduction of techniques such as digital processing of images taken from the microscope or directly from the organisms could significantly reduce the time required to obtain data in ecological studies (Álvarez-Borrego et al., 2002). Several successful digital correlation methods for image processing have been developed based on the shape of the organism of interest (Pech-Pacheco and Álvarez-Borrego, 1998). While this method has been primarily used to identify species in multispecies assemblages, increasing the resolution and inclusion of particular phenotypic characters can also permit the recognition of an individual within a group or population.

Table 2. Values of percentage correlation when comparing pre-molt to post-molt images of individuals; boldface numbers correspond to the value of intra-individual correlation while regular numbers represent the values of inter-individual correlation, i.e., the pre-molt S_{POF} of each individual (at start of experiment) is compared with the post-molt S_{POF} of itself (in boldface) and all 13 other individuals (in regular type face), i.e., correlation values for each individual are read in the lines from left to right (see 'Materials and methods' for explanation of terms).

	Post-molt image of individuals													
Ind	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Pre-molt image of focal individual														
1	**21.2**	17.0	13.3	19.4	16.6	19.4	11.9	14.3	11.2	17.8	15.4	12.6	15.5	16.3
2	8.4	**39.8**	8.8	13.3	13.5	14.2	11.8	9.9	12.2	12.0	9.6	8.6	10.6	10.0
3	8.8	11.8	**26.0**	13.1	17.5	22.6	9.1	16.7	11.4	15.4	11.5	15.8	16.1	14.9
4	10.2	13.8	11.5	**22.0**	13.8	16.4	8.2	15.0	11.5	12.3	9.6	16.6	12.5	14.5
5	10.8	7.1	6.9	12.5	**20.3**	10.8	11.7	6.5	11.3	7.4	11.3	7.4	8.0	8.6
6	15.7	13.3	12.7	18.9	23.3	**35.3**	13.7	12.2	12.6	15.4	14.2	11.5	14.6	16.4
7	9.4	9.4	11.6	10.7	12.3	12.5	**20.3**	12.4	11.5	7.4	10.0	9.0	9.4	9.0
8	11.2	10.7	15.5	14.2	13.0	15.2	12.3	**17.5**	12.6	10.2	9.5	15.9	14.5	12.6
9	9.8	8.6	7.4	19.0	13.7	16.8	16.1	8.8	**24.6**	9.1	8.5	10.1	13.8	13.4
10	6.7	7.9	5.2	9.3	7.8	8.8	6.9	6.5	6.1	**10.2**	5.7	5.8	7.0	6.7
11	11.3	11.8	10.7	14.0	13.7	18.6	10.5	9.0	9.0	10.5	**25.3**	7.8	7.6	8.5
12	14.1	12.7	11.4	16.4	14.3	15.9	16.5	12.8	13.0	12.7	14.5	**20.4**	12.6	11.3
13	12.4	12.2	12.2	12.5	12.3	12.0	12.1	12.2	12.6	12.4	12.7	12.6	**18.8**	10.8
14	15.5	14.1	13.9	12.9	11.3	11.4	12.1	12.5	12.9	13.9	11.7	11.9	12.9	**23.1**

In the present study, natural body marks (here color patterns) allowed us to discriminate among individuals and to track these beyond one molt. Our results demonstrated that rock shrimp had a relatively homogenous coloration pattern of 83.1% similarity among shrimps, i.e., exhibited a mean intra-specific variation of only 16.9%. We are not aware of other studies examining the degree of similarity in color patterns within a population of crustacean conspecifics, but we suspect that other highly colorful shrimp (for examples see Bauer, 2004) may exhibit similar intra-specific variation in color patterns. Color patterns could thus be an interesting tool to distinguish between individuals in those species that do not feature short-term color changes. The mean intra-specific variation of 16.9% was sufficient to discriminate among the 14 individuals we tested. However, the low degree of variation among individuals may not allow individuals to be distinguished in larger groups or in natural populations. The discriminatory power of this method could in the future be improved by incorporating additional characters, e.g., images from other body regions, increasing its applicability to situations with considerably larger numbers of conspecifics.

Our results also showed that the general color patterns persisted after one molt. In all cases, the intra-individual correlation values were greater than the inter-individual correlation. However, for one individual (#10) the differences between the intra- and some inter-individual correlation values were small. Methodological problems most likely were responsible for this as upon post-hoc revision we noticed that the first image taken of this animal was not fully centered in the focal plane. This may also have contributed to the fact that the premolt image of this individual had a relatively non-specific color pattern when compared to the other 13 individuals (see also Table 1). Future efforts should thus also focus on the improvement of the method. The proposed algorithm could be improved in order to calibrate the images with respect to their position, scale, and rotation. This point is highly critical in mathematical correlation because changes in position, scale or rotation are reflected as changes in the correlation plane (for details see Pech-Pacheco et al., 2001b).

To our knowledge few studies have been carried out to identify crustacean individuals based on natural body marks. Recently, MacDiarmid et al. (2005) described the conservation of unique patterns of body marks in individuals of the spiny lobster *Jasus edwardsii*. Color shadings and spine patterns varied uniquely among individuals, and marks are retained after molting allowing recognition of individuals. Distinct marks in the facial region and on the epistoma, as well as the number and placement of small spines on the antennular plate are particularly useful for recognizing lobsters (MacDiarmid et al., 2005). Many crustaceans have dense groups of spines on their carapace, and a study by Gosselin et al. (2007) on *Chionoecetes opilio* confirms the usefulness of this character in tracking individuals over time. Our study proposes an alternative method to recognize crustaceans by using correlation of diffraction patterns (CDP). The advantage of this method is the rapid and accurate data processing, and the possibility of digital storage in a database against which additional sample images can be compared. This sort of database and image analysis, when further improved, e.g., by including additional characters such as spines, natural body marks, or images from other body regions, might become useful in ecological studies or tracking programs of several conspecifics. Many (but not all) crustacean species feature distinct color and spination patterns that could be useful for individual identification. Future studies are necessary to determine whether body marks are individual-specific and whether they are conserved over one or more molts, in order to permit long-term tracking of individuals.

Acknowledgements

We are grateful to I. Hinojosa, P. Bravo and E. Macaya for help with capture, maintenance and photographing of shrimp. Constructive comments by T. Gosselin, B. Sainte-Marie and by two anonymous reviewers helped to improve the manuscript.

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RECEIVED: 12 June 2006.
ACCEPTED: 31 October 2006.