Proceedings of the 11th International Coral Reef Symposium, Ft. Lauderdale, Florida, 7-11 July 2008
Session number 22

Photo-ID on reef fish – avoiding tagging-induced biases

M. Perrig, B.P.L. Goh

Natural Science and Science Education, National Institute of Education, Nanyang Technological University, 1 Nanyang Walk, Singapore 637616

Abstract. Mark-recapture experiments are essential to estimate population parameters. However, physical tagging is often biased, potentially affecting mortality rates, performance, and behavior. Photo-ID is an unbiased alternative commonly used on marine mammals, but has barely been used on teleost fishes. This study examined the individuality and stability of coloration patterns of two rabbitfish species (*Siganus javus* and *Siganus guttatus*) to determine if photo-ID could be extended to teleost fish. Examination of 189 *S. javus* and 34 *S. guttatus* revealed no duplicated coloration pattern within both species. The coloration patterns of six sub-adult *S. javus* and four adult *S. guttatus* remained easily recognizable over two and eight months, respectively. The results of this study suggest that the coloration patterns of *S. javus* and *S. guttatus* are a reliable means to recognize individuals over time, thus allowing unbiased mark-recapture studies on two coral reef fish species.

Key words: photo-ID, *Siganus javus*, *Siganus guttatus*, coloration pattern

Introduction

Assessing the status of populations and managing them as resources heavily rely on the knowledge of population size and parameters influencing it, namely the BIDE parameters (birth, immigration, death, and emigration; Williams et al. 2002). Many of these parameters can be determined via mark-recapture experiments (Amstrup et al. 2005). However, physically marking animals with tags may have negative impacts on the parameter to be estimated, affecting the reliability of the results (Thorsteinsson 2002). For example, stress due to capture and handling, infection risks, and changed performance may increase post-tagging mortality and therefore bias estimated survival rates (Thorsteinsson 2002).

Mark-recapture experiments using photographic identification (photo-ID) forego tagging induced biases (Amstrup et al. 2005). This technique determines encounter histories by recognizing animals based on naturally occurring morphological features, such as scars, shapes, or coloration patterns (e.g. Speed et al., 2007; van Tienhoven et al., 2007), thus avoiding physical tagging altogether.

This approach has two major underlying assumptions: 1) every individual of a population is sufficiently different from other individuals that they are not misidentified; and 2) features or coloration patterns do not change over the time scale of the study.

It is generally accepted by the research community that no individual is completely identical to its conspecifics. Very important theories are based on such individual variation (e.g. Darwin's evolutionary theory; Campbell, 1995). However, phenotypic differences can be subtle. In practical terms for photo-ID, two coloration patterns could be similar enough to appear identical to an observer, thus leading to misidentifications.

In contrast to generally accepted individuality, the stability of coloration patterns is a major concern using photo-ID. In order to reliably identify an animal, the pattern should not change over the time course of the study. In many fish species, this can potentially pose a problem: Ontogenetic shifts, mood changes as well as growth can induce changes in the coloration pattern (Allen 2002). Alterations due to mood changes are usually very obvious and of little impact to mark-recapture studies.

More important are gradual changes associated with the growth of an animal. Patterns do not necessarily grow in proportion with the body. Gradually expanding patterns by the addition of marks or splitting of dots or lines is also possible. Leopard sharks (*Stegostoma fasciatum*), for example, loose their stripes by dissolving them into spots and rings while growing (Mahon J. L., pers. comm.). Because this is normally a slow and gradual process, it is easily overlooked and therefore may pose a major challenge in photo-ID. Over time, the coloration pattern of an individual might change enough such that it is no longer identified as the same animal.

Photo-ID has been successfully applied on many large and long-lived species, typically marine mammals (e.g. Caswell et al., 1999; Neumann et al. 2002; Langtimm et al., 2004). However, application of photo-ID is by no means restricted to such species.

For example, with their highly ornamented coloration patterns many coral reef fishes are good candidates for photo-ID. Nonetheless, this non-intrusive technique has barely been applied to teleost fish, and data that is unbiased by physical tagging is rare.

This study aimed to extend photo-ID as a reliable technique to two herbivorous fish species on coral reefs: *Siganus javus* and *Siganus guttatus*. The coloration of members from both species was examined over time to establish the temporal extent to which the coloration patterns of these two species could be used as reliable identifiers. Furthermore, patterns were also examined to determine their use for individual identification, and to determine the frequency of coloration duplication. The right and left sides of individuals were also compared to confirm the absence of symmetry.

Materials & Methods

Individuality

A total of 189 *S. javus* and 34 *S. guttatus* were photographed on their right and left sides. Photographs were taken approximately perpendicular to the side of the animal, both from live and dead fish. Live animals were photographed in a small aquarium with little interference. The shape and relative position of dots and lines on the entire body of the fish (excluding fins and head) within each species and side were manually compared with each other. The right and left sides of each individual were also compared with each other.

Comparisons were classified as 'identical', 'different', or 'undetermined'. Two photographs were defined as 'different' if three or more differences in coloration pattern could be detected. Instances where photograph quality, reflections, or abrasions prevented comparison over more than approximately 75% of the fish body were classified as 'undetermined'.

Stability

A school of sub-adult *S. javus* was held in the quarantine area of Underwater World Singapore (UWS) and was monitored over time. Throughout the study, a total of 12 animals were fin-clipped in different combinations in the posterior part of the anal, dorsal, and caudal fins for individual recognition within a common tank. The total length of each fish was also measured. In contrast, only four adult *S. guttatus* were available in a large exhibit at UWS. All photographs were taken with minimal interference, i.e. by patiently waiting outside the tank for the animals to swim by. Only photographs approximately at a right angle from the body surface were selected.

Comparing two photographs from the same side and individual, the number of changes was recorded. Splits, fusions, appearances, or disappearances of dots or lines were all treated identically. Right-sided and left-sided photographs were analyzed separately.

<u>Siganus javus</u>

Changes over hours: Seven animals were monitored over eight hours of a day to check for short-term changes in coloration patterns. They were photographed on both sides within 30 minutes, at 8am, 10am, 12noon, 2pm, and 4pm. This experiment was repeated on four days (September 19, October 17 and 24, and November 7, 2007). Photographs were manually compared to detect any changes over two-, four-, six-, and eight-hour intervals.

Changes over days: Similarly, photographs from 23 days were used to examine the variation in coloration patterns of 12 animals over one to six days.

Changes over weeks: Photographs from October 24, 2007 were compared to photographs taken one, two, three, four, and five weeks later. A total of 11 fish were monitored in this way.

Assuming that changes in coloration patterns occur gradually and at a constant rate over weeks, averaging changes over several consecutive equal time periods is more representative for a particular side of a fish. For the different weekly intervals (i.e. one- to five-weekly), non-overlapping intervals were compared and averaged to represent the rate of change.

Long-term changes: Photographs of six fish on each side were available for comparisons over the two months from September 27, 2007 to November 28, 2007 (62 days). Only a single fish was monitored over more than two months. The photo series of this animal covered 161 and 168 days on the right and left side, respectively. It was used to track changes and document their appearance on a time scale.

<u>Siganus guttatus</u>

Four *S. guttatus* were photographed at irregular time intervals from September 4, 2007 to May 5, 2008 (244 days; approximately 8 months). The best photographs taken on the first and last day of observation were compared to determine changes that may have appeared on each side of each fish. The photo series were examined to determine the first appearance of each change.

Statistical analysis

Working with count data, errors are not normally distributed and therefore, two samples were compared with the non-parametric Wilcoxon rank sum test (Crawley 2005). Program R was used to conduct the analysis (The R Foundation for Statistical Computing 2007).

Results

Individuality

The relation of six (0.03%) and nine (0.05%) comparisons of *S. javus* on the right and left side, respectively, could not be determined due to photograph quality, reflections or abrasions. No comparison resulted in an 'identical' result (Fig. 1). Every comparison of *S. guttatus* was found to be different (Fig. 1). All 189 individual *S. javus* and all 34 individual *S. guttatus* differed in their coloration patterns on their right and left sides.

Figure 1: Examples for distinctively different coloration patterns of *S. javus* (top) and *S. guttatus* (bottom). Differences in *S. javus* were most evident at the posterior part below the dorsal fin, while differences in *S. guttatus* were more distinctive behind the gill cover.

Stability

Changes in coloration pattern occurred gradually over time (Fig. 2). They occurred faster in sub-adult *S. javus* than in adult *S. guttatus*.

<u>*Siganus javus*</u>

Fish were sized from 14 cm to 19 cm at the time of fin-clipping. The first differences in coloration patterns of *S. javus* occurred in weekly intervals. No changes were observed within time intervals of less than one week.

Changes over weeks: Within all one- to five-weekly intervals only three out of 12 fish showed some changes. Over one-, two-, and three-weekly intervals no more than a single change per side and fish was observed. A maximum of two changes per side and per fish was found in four- and five-weekly intervals.

The number of changes observed on the right and left sides were not significantly different ($p > 0.3404$). Single measurements from the same date and averaged measurements from consecutive intervals showed similar results for one- to five-weekly periods ($p > 0.1675$). The number of changes was not significantly different from zero ($p > 0.3458$).

Long-term changes: Over two months, only two out of six fish accounted for all eight changes (one fish had two changes per side, the other one showed four changes on the right side). The numbers of changes were not significantly different from zero ($p > 0.3711$), and did not differ between the right and the left sides ($p = 0.5271$).

Over longer intervals, 16 differences on the right side over 161 days and 13 differences on the left side over 168 days were recorded from a single *S. javus* monitored. Averaged over approximately 5.5 months, this corresponded to a monthly changing rate of 2.9 and 2.4 changes per month (CPM) on the right and left sides, respectively. Changes gradually accumulated over time.

Despite the changes, individual *S. javus* could easily be recognized after two months, including the side of one fish that underwent four changes. However, after 161 and 168 days (app. 5.5 months) on the right and left sides, respectively, discrepancies made recognition less easy, i.e. manual matching would most likely have failed on the right side.

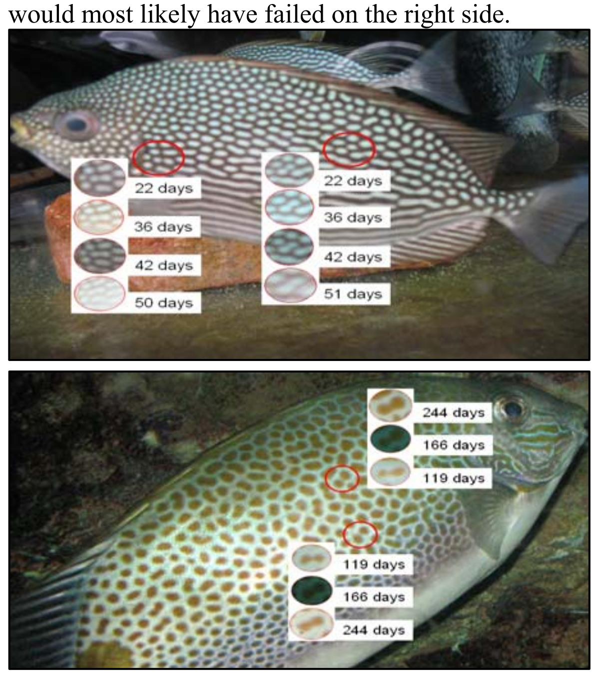

Figure 2: Gradual splitting dots in *S. javus* (top) and *S. guttatus* (bottom). The number next to the circles indicates how many days earlier the picture of this section was taken. Note that the splitting process was much faster in the sub-adult *S. javus* compared to the adult *S. guttatus*.

<u>*Siganus guttatus*</u>

On the right and left side, the four animals experienced on average 3.5 and 2.75 changes, respectively, over the 244-day observation period.

This was equivalent to 0.4 and 0.3 CPM. Early in the observation period, one fish obtained a superficial scratch on the right side, which healed in the time course of the study. The pattern at the location of the scratch remained largely unchanged. Only one dot stretched into a short line. Over the entire period, all four animals could very easily and unequivocally be recognized through photographs of both sides. The number of changes on the right and left sides were not significantly different (p = 1).

Discussion

Coloration patterns as individual identifiers

According to intuitive expectations, neither *S. javus* nor *S. guttatus* showed symmetrical coloration patterns. Consequently, photographs taken from the right and left side of an individual failed to match and the two datasets had to be treated separately in mark-recapture studies. Schwarz C. J. (pers. comm.) suggested treating the two datasets as independent sampling methods of the same population.

With no similar coloration pattern found twice within both sides of both species, the data suggests that twin-patterns can be practically excluded. This supports the intuitive assumption taken by many researchers when applying photo-ID: every animal has unique coloration, markings, and features. However, results should not be over-weighted. These schooling fish species occur in large parts of South-East Asia (*S. guttatus*) and, for *S. javus*, stretches as far as Australia and the Persian Gulf (Woodland, 1990). The sample sizes of 34 and 189 specimens of *S. guttatus* and *S. javus* used in this study, respectively, make up a very small sample to consider. Nevertheless, it seems reasonable to assume that twin-patterns do not occur in significant numbers in populations of *S. javus* and *S. guttatus*.

Stability of coloration patterns

Intuitively, one would anticipate coloration patterns to change gradually in relation to growth rates, i.e. patterns are expected to change equally on both sides of the animal and to do so mostly during fast growing stages of the life cycle. Stages with little or no growth are believed to exhibit very stable coloration patterns.

The results of this study support this intuitive theory. Changes occurred gradually and in equal quantities on the right and left side in both species. Assuming that *S. javus* and *S. guttatus* both follow the Von Bertalanffy growth curve, which a majority of marine organisms do (e.g. many elasmobranches; Cailliet et al., 1992; van Dykhuizen and Mollet, 1992; Acanthuridae; Choat and Axe, 1996; Scaridae; Choat et al., 1996), juveniles and sub-adults would be expected to grow fast compared to adults. If changing rates are indeed positively correlated to growth rates, coloration patterns would be expected to also change faster in small fish. According to Woodland (1990), *S. javus* commonly measure less than 30 cm standard length (SL), but they can grow considerably larger. With a maximum of 19 cm total length (TL), the fish used in this study are thus believed to reflect the sub-adult, fast-changing part of the life cycle.

In contrast, the four *S. guttatus* at UWS were fully-grown adults. They have been kept for at least one year prior to the start of the observations (pers. obs.). Although no measurement could be taken, animals were estimated to be close to 30 cm SL in July 2007. With a maximal SL of 35 cm reported by Woodland (1990), it is reasonable to assume that the animals at UWS represent the slow-growing and thus, the slow-changing phase of their life cycle.

In accordance with the intuitive theory, the sub-adult *S. javus* monitored over more than five months showed a much larger change in coloration pattern than did *S. guttatus* over eight months. With 2.9 changes per month (CPM) on the right side and 2.4 CPM on the left side *S. javus* changed at a much faster rate than *S. guttatus*, which averaged 0.4 and 0.3 CPM on the right and left side, respectively. After five months, *S. javus* could no longer be unambiguously identified, while *S. guttatus* were easily recognized even after eight months.

Although growth pattern and changing rates are not generally comparable among different species, they can be assumed to be similar in the two species of interest here. Both species show many anatomical similarities such as spine arrangement, jaw structure, and fright pattern (Woodland, 1990). They both grow to a comparable size, and have similar patterns consisting of dots and lines (Woodland, 1990). Furthermore, they are genetically very closely related (Lemer et al. 2006).

The results from sub-adult *S. javus* suggest that patterns only remain entirely stable on a very short time scale. After six days, changes started to occur in some fish, but animals remained easily recognizable even after two months, with up to four discrepancies per side. Therefore, photo-ID can be reliably used on *S. javus* up to 62 days. The fact that the number of changes was not significantly different from zero over all time intervals up to two months supports this conclusion.

The two-month reliability for photo-ID of sub-adult *S. javus* is a conservative estimation. The single fish monitored for a longer period showed only six changes after 134 days on the left side. Over both intervals, it was still possible to identify the two photographs as taken from the same individual, suggesting that photo-ID could also be used over time periods of up to four months. Likewise, the right side remained easily identifiable over 127 days despite 13 changes. Identification of individuals on both sides only became difficult after more than a five-month interval.

In contrast, the adult *S. guttatus* exhibited much more stable coloration patterns. After the entire observation period of 244 days (app. 8 months), only very few changes occurred. All animals remained unmistakably identifiable. Thus, mark-recapture studies using photo-ID can be reliably used on adult *S. guttatus* over at least eight months.

To apply these results mechanically to all populations of *S. javus* and *S. guttatus* and its members may lead to inaccuracies. The two-month stability observed in sub-adults of *S. javus* is likely to be a more accurate estimate for sub-adult *S. guttatus* than the eight-month stability determined for adult *S. guttatus*. Identification of a sub-adult, presumably fast-growing *S. guttatus* after eight months is likely to fail and thus may lead to unrepresentative data. Similarly, adult *S. javus* may exhibit a stable pattern similar to that of adult *S. guttatus*.

The reliable identification intervals found in this study contrast with conventional tagging. The latter is often marked by a high rate of tag loss and tagging induced mortality shortly after release, followed by a period of lower loss rates (Thorsteinsson 2002). In contrast, coloration patterns are not lost and change only gradually. Thus, photo-ID studies are most reliable directly after the initial photographic capture and can potentially generate more accurate data in this regard.

Extraction of encounter histories from photographs is a tedious process, a disadvantage compared to physical tagging. However, the use of new software can accelerate the comparison of photographs (Speed et al. 2007; van Tienhoven et al. 2007). Furthermore, photo-ID is preferable when capturing live animals is difficult or when mortality rates have to be kept at zero (e.g. for species at risk).

While it is relatively easy to conduct photo-ID studies in captivity, obtaining photographs in the wild may pose a problem for non territorial and schooling fishes. However, photo-ID was still successfully used by the author to estimate short-term site-fidelity and localized population sizes of *S. guttatus* and *S. javus*. Territorial non schooling fishes (i.e. many Serranidae) promise more extensive applications of photo-ID.

In conclusion, the data suggests that photographs of individual sub-adult *S. javus* and adult *S. guttatus* can be reliably recognized over at least two and eight months, respectively. Within these conservative limits, photo-ID is believed to yield very accurate results for the two species: *S. javus* and *S. guttatus*.

Acknowledgement

The authors express their many thanks to Underwater World Singapore for kindly letting us use their facilities. This project was funded by National Institute of Education / Nanyang Technological University Grant (AcRF RP 5/02 GPL) and Singapore Institute of Biology Research Trust Fund (RTF 033/2007). The project was conducted under the NTU-IACUC Permit (ARF SBS/NIE-A 0031).

References

Allen GR (2002) Marine fishes of the Great Barrier Reef and South-East Asia. Western Australian Museum. Sydney.

Amstrup SC, McDonald TL, Manly BFJ (2005) Handbook of Capture-Recapture Analysis. Princeton University Press. Woodstock. 296 pp.

Cailliet GM, Mollet HF, Pittenger GG, Bedford D, Natanson LJ (1992) Growth and demography of the pacific angel shark (*Squatina californica*), based upon tag returns off California. Aust J Mar Freshw Res 43:1313-1330.

Campbell NA (1995) Biologie. De Boeck Université. Paris. 1190 pp.

Caswell H, Fujiwara M Brault S (1999) Declining survival probability threatens the North Atlantic right whale. Pop Biol 96:3308-3313.

Choat, J. H. and Axe, L. M. 1996. Growth and longevity in acanthurid fishes; an analysis of otolith increments. Mar Ecol Progr Ser 134:15-26.

Choat JH, Axe LM Lou DC (1996) Growth and longevity in fishes of the family Scaridae. Mar Ecol Progr Ser 145:33-41.

Crawley MJ (2005) Statistics, an introduction using R. Wiley. West Sussex. 342 pp.

Langtimm CA, Beck CA, Edwards HH, Fick-Child KJ, Ackerman, BB, Barton SL, Hartley WC (2004) Survival estimates for Florida manatees from the photo-identification of individuals. Mar Mammal Sci 20 (3), 438-463.

Lemer S, Aurelle D, Vigliola L, Durand J-D,Borsa P (2006) Cytochrome b barcoding, molecular systematics and geographic differentiation in rabbitfishes (Siganidae). C. R. Biologies doi:10.1016/j.crvi.2006.09.002.

Neumann DR, Leitenberger A, Orams MB (2002) Photo-identification of short-beaked common dolphins (Delphinus delphis) in north-east New Zealand: a photo-catalogue of recognisable individuals. NZ J Mar Freshw Res 36, 593-604.

Speed CW, Meekan MG, Bradshaw CJA (2007) Spot the match – wildlife photo-identification using information theory. Frontiers in Zoology 4 (2), doi:10.1186/1742-9994-4-2.

The R Foundation for Statistical Computing. 2007. www.r-project.org/foundation/. Accessed 25th of June 2008, 12.56.

Thorsteinsson V (2002) (Co-ordinator). Tagging methods for stock assessment and research in fisheries. Report of Concerted Action: FAIR CT.96.1394 (CATAG). Marine Research Institute, Reykjavik, Iceland.

van Dykhuizen G, Mollet HF (1992) Growth, age estimation, and feeding of captive sevengill sharks, *Notorynchus cepedianus*, at the Monterey Bay Aquarium. In: Pepperell J. G. (ed) Sharks. Biology and Fisheries. Aust J Mar Freshw Res 43: 297-318.

van Tienhoven AM, den Hartog JE, Reijns RA. Peddemors VM (2007) A computer-aided program for pattern-matching of natural marks on the spotted raggedtooth shark *Carcharias taurus*. J Appl Ecol 44, 273-280.

Williams BK, Nichols JD, Conroy MJ (2002) Analysis and management of animal populations. Academic Press. London. 1040 pp.

Woodland JD (1990) Indo-Pacific fishes. Revision of the fish family Siganidae with descriptions of two new species and comments on distribution and biology. Bernice Pauahi Bishop Museum, Honolulu, Hawaii.