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Partial fin-clipping as an effective tool for tissue sampling seahorses, *Hippocampus* spp.

LUCY C. WOODALL[1,3], RACHEL JONES[2], BRIAN ZIMMERMAN[2], SAMANTHA GUILLAUME[2], TEAGUE STUBBINGTON[2], PAUL SHAW[1] AND HEATHER J. KOLDEWEY[2]

[1]Zoology Department, The Natural History Museum, Cromwell Road, London SW7 5BD, [2]Zoological Society of London, Regent's Park, London, NW1 4RY, [3]University of Stirling, Stirling, Scotland, FK9 4LA

*Partial fin-clipping is a non-lethal sampling technique commonly used to sample tissue for molecular genetic studies of fish. The effect of this technique was tested on seahorses (*Hippocampus *spp.) as they have several peculiar biological characteristics when compared with other fish and are on the* IUCN Red List of Threatened Species. *Partial fin-clipping of the seahorse dorsal fin was evaluated on* Hippocampus kuda. *The fish were assessed for short-term effects (fin re-growth time) as well as the longer term effects (growth and mortality) of partial fin clipping over a four month period. Total fin re-growth occurred between 2 and 4 weeks with no significant difference observed in the fin re-growth time between sexes. There was no significant difference between the mortality rate/growth rate of clipped versus unclipped seahorses. Results indicate partial fin-clipping has no significant effect on seahorses, and should be considered as a useful method for tissue sampling.*

Keywords: partial fin-clipping, seahorse, *Hippocampus kuda*, sampling, genetics

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INTRODUCTION

The field of conservation genetics has developed as an interdisciplinary science that applies genetic methods to the conservation and restoration of biodiversity. One challenge for such studies is the collection of tissue suitable for DNA analysis from endangered and/or threatened species. Lethal sampling is usually inappropriate, illegal or impossible, forcing researchers to use more inventive non-destructive methods to obtain samples.

Molecular methodology is considered an essential technique for marine conservation. Conservation genetics enables practitioners to understand species' movement patterns, population connectivity, breeding success, mating systems and to develop/manage captive breeding programmes (Schwartz *et al.*, 2007). Molecular studies require a sample of tissue from the target species. However the sample only needs to be very small, as the polymerase chain reaction (PCR) laboratory technique exponentially amplifies the region of target DNA. The DNA obtained from partial fin clipping is enough for tens of reactions. For small fish there are a limited number of possible non-lethal sampling methods, including removal of scales, blood, barbels, sperm, epidermal mucous or buccal tissue (Campanella & Smalley, 2006; Lucentini, *et al.*, 2006), but the method most widely used is partial fin-clipping.

For many years the total or partial removal of fins has been successfully applied as a fish-marking technique within fisheries management (Ricker, 1949). Studies employing this technique have looked at fish growth (e.g. Coble, 1971), movement (e.g. Crawford, 1958) and survival (e.g. Gjerde & Refstie, 1985), in order to estimate population size and to monitor stocking (Pratt & Fox, 2002). More recently, with the increased popularity of DNA-based analysis for ecological and fisheries studies (O'Reilly & Wright, 1995), partial fin-clipping has been employed for tissue collection purposes when non-lethal methods are required (Wasko *et al.*, 2003).

One study to date has expressly addressed the effect of partial fin-clipping on fish. This study focused on freshwater fish and showed no significant difference in growth rate between clipped and unclipped fish, it also showed fin regeneration within 12 weeks. A number of other studies, to determine the usefulness of total and partial fin-clipping for tagging fish, have mostly tested freshwater or anadromous species. These studies found no significant effect on fish growth or mortality in partially fin-clipped fish (Coble, 1967; Gjerde & Refstie, 1985; Basavaraju *et al.*, 1998; Conover & Sheehan, 1999; Thompson *et al.*, 2005; Johnsen & Ugedal, 2008). Additional studies observed complete fin regeneration for partially clipped fins and also complete fin removal (Weber & Wahle, 1969; Schulz, 1997; Diekes *et al.*, 1999; Katano & Uchida, 2006; Champagne *et al.*, 2008). Even though fin clips are a common method of tissue collection in marine fish, all studies examining the effect of fin-clipping have focused on just a few fish species which are fusiform and none of which are exclusively marine.

Seahorses are unusual fish in that they have an upright posture, small fin size, skin and bony plates instead of scales and prehensile tails that replace the caudal fin (Foster & Vincent, 2004). Seahorses are largely sessile, with their small fins used for propulsion (dorsal fin) and orientation (pectoral fin) (Foster & Vincent, 2004). Additionally the seahorse possesses a tiny anal fin which does not have an obvious function (Foster & Vincent, 2004). The small fin size of seahorses may

Corresponding author:
L.C. Woodall
Email: l.woodall@nhm.ac.uk

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suggest they are unsuitable candidates for fin-clipping. However, dependency on museum samples or dried specimens would considerably constrain the potential to answer many questions relating to seahorse conservation, from species identification to population structure.

To date, partial fin-clipping of live seahorses has been used as a tissue sampling method for several genetic studies (Lourie & Vincent, 2004; Teske *et al.*, 2004, 2005; Lourie *et al.*, 2005; Sanders *et al.*, 2008). The use of partial fin-clipping on seahorses has been assessed in two trials (Lourie, 2003). The first of these was limited by sample size (N = 3), but no mortality was observed for *Hippocampus erectus* after one month. In a second, larger experiment (N = 100) over two months on *Hippocampus kuda*, no conclusions could be drawn due to tank effect and the effect of the tagging method; however, 14% of the seahorses appeared not to recover from the partial fin-clipping (S. Lourie, personal correspondence). To date, much knowledge on the effect of partial fin-clipping on seahorses is anecdotal, small-scale and inconclusive. Yet this method is being increasingly applied. Therefore it is important to evaluate it fully.

This study aimed to conduct a comprehensive assessment of partial fin-clipping as a viable method for use on seahorses, particularly considering their conservation status. To achieve this, we tested the short and long-term effects of partial fin-clipping on seahorses by assessing growth, mortality and fin re-growth. To test whether partial fin-clipping produces sufficient DNA for subsequent molecular analysis we extracted DNA from this fin-clip.

MATERIALS AND METHODS

This study was subject to the Zoological Society of London (ZSL) Ethics Committee, and all procedures were approved and licensed by the British Home Office Animals Scientific Procedures Act 1986. The study was conducted under personal licence (PIL80/10005) and project licence (PPL80/2043).

Thirty-six *Hippocampus kuda* were randomly selected from a population of 50 captive-bred seahorses held at the Blue Reef Aquarium, Newquay, and transferred to a specially designed tank at the ZSL Aquarium. The adult seahorses (18:18), were all kept in one tank (720 litres of natural seawater), and were provided with holdfasts (dead coral) to normalize behaviour. The tank had a light regime of 12:12 photoperiod using a 36W white fluorescent tube, was heated to 26.5–28.0°C using two Jaeger 300W stick heaters, and had an external filtration system comprising three parts: an Eheim external canister filter 2260, filled with biological media and floss (Stalybridge, UK); X2 Venturi Protein Skimmer; and a 25W Vectron UV sterilization unit. Throughout the experiment, the seahorses were fed three times a day with *Mysis rellicta*, *Artemia salina* and *Euphasia pacifica* enriched with Zoolife® fishvits (Escondido, USA). Once stabilized the tank water was maintained within the following parameters: pH 7.8–8.0; $CaCO_3$ 80–105 ppm; NH_3 0–0.22 ppm; NO_2 0.01–0.13 ppm; NO_3 2–10 ppm; and salinity 32.9–34.8 ppt.

The experiment was conducted over 22 weeks. After an initial stabilization period of six weeks following transfer to the new tank, all seahorses were tagged with visible implant fluorescent elastomer (VIFE) (Northwest Marine Technology Inc.) marking different body segments to identify individuals, this ensured no handling was required for subsequent identification (Woods & Martin-Smith, 2004; Curtis, 2006). Following a two week recovery (week 8) the seahorses were weighed and straight trunk length (TL) measured (Curtis & Vincent, 2006), then randomly assigned into two groups (clipped and unclipped). A meshed partition was placed in the tank, the fin-clipping took place on one side of the tank and the remaining fish were on the other. The fin-clipping procedure was conducted as follows: the experimenter, while wearing gloves, held the seahorse with one hand and using toe-nail clippers sampled approximately 2 mm^2 of tissue from the lower part of the dorsal fin (Figure 1). When all fish from the clipped group were sampled, the meshed partition was removed. The tissue samples were then stored in absolute ethanol.

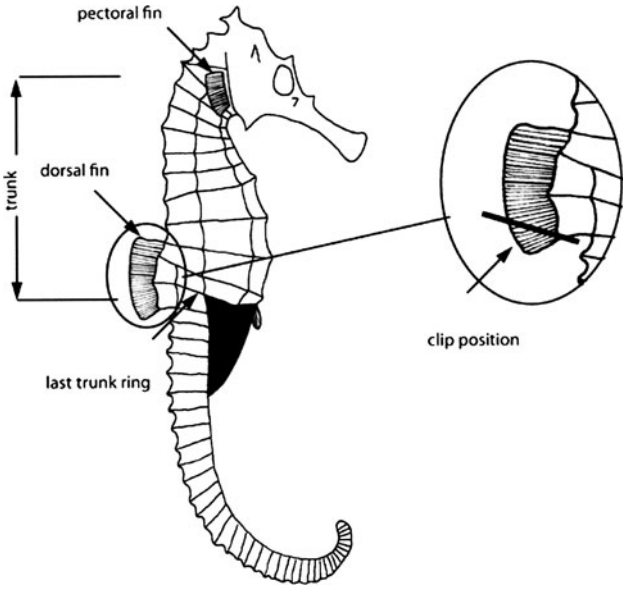

Fig. 1. Seahorse *Hippocampus kuda* with dorsal fin tissue clipped (adapted from Lourie et al., 1999).

The seahorses were monitored daily for signs of ill health by the expert aquarists of the ZSL Aquarium. To assess short-term effects of partial fin clipping, the fin re-growth was recorded weekly until total fin re-growth in all clipped seahorses was observed (week 12 of the experiment). The coding used for fin re-growth was designed so that seahorses did not need to be handled and to minimize observer bias (Table 1).

The long-term effects of partial fin-clipping were assessed by comparing seahorse mortality, disease outbreaks and growth rates, between clipped and unclipped groups. Growth rates were determined by taking monthly measurements of TL and weight (weeks 8, 12, 16 and 22 of the experiment). The final measurements were delayed for 2 weeks, so

Table 1. Coding for amount of seahorse dorsal fin re-growth.

Amount of dorsal fin re-growth	Description of fin	Code
Complete	No difference seen between clipped and unclipped fin	3
Near complete	90% re-growth of fin	2
Partial	Any re-growth observed	1
None	No re-growth seen	0

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that they would coincide with a tank move, required by ZSL. This delay was to avoid unnecessary stress caused by double handling within a month. Throughout the experiment, details of any injury, disease, death or pregnancy were recorded on a daily basis. In most cases these observations could be assigned to individuals through identification of VIFE tags, but on some occasions this was not possible, e.g. when an individual could not be identified without handling (some seahorses preferentially stayed within the holdfast structures thereby making VIFE tag observations difficult or impossible).

The data collected were analysed in two parts to evaluate the effects of fin clipping on seahorses:

(i) Short-term effects

Fin re-growth period. Fin re-growth was assessed using a two sample *t*-test to determine if there were differences in re-growth time between males and females, and a Mann–Whitney test on re-growth category at the second week after clipping (week 10) to investigate differences between sexes. The re-growth at week 10 was chosen as that was the first week that total re-growth was observed in an individual.

(ii) Long-term effects

Individual growth rate (TL and weight) was recorded to assess long-term effects. A generalized linear model (GLM) was used to ascertain the long- term effects of fin-clipping. The parameters used were: sex, clipped or control (unclipped) fish, time of TL and weight measurement and initial measurement of TL and weight. These parameters were assessed in combination (i.e. female clipped versus male control) and individually to determine their effect on final TL and weight. Only individuals with all measurements were included in the analysis. All statistical tests were conducted in Minitab 16.1.1 (Minitab Inc.).

DNA was isolated from less than half of the fin-clip preserved in ethanol using a modified cetyltrimethylammonium bromide (CTAB) extraction protocol (Winnepenninckx *et al.*, 1993). The extracted DNA was run on a 1% agarose gel ethidium bromide stained gel and visualized in UV light.

RESULTS

All partially fin-clipped seahorses showed complete fin re-growth after four weeks (week 12), with no difference in growth rate between clipped and unclipped seahorses. In addition, no mortalities were observed that could be attributed to the fin-clipping procedure.

Short-term effects

There was no significant difference in total fin re-growth time between males and females ($t = -1.51$, $P = 0.150$, df = 16). Fin re-growth was quick, with some individuals having noticeable re-growth after just one week and complete re-growth in all seahorses in four weeks. The time taken for total re-growth of the seahorse dorsal fin was, on average, three weeks (week 11) (mean 3.22 weeks, SD 0.647), when 66% of seahorses showed full re-growth. Two weeks after fin-clipping (week 10) there was the biggest difference in the average re-growth code between males and females, but this difference was not significant ($\chi^2 = 2.67$, $P = 0.26$, df = 2).

Table 2. The effect of sex, clipped/control and time of measurement, on the length and weight of *Hippocampus kuda* in the fin clipping trials. Values significantly greater than zero are indicated with asterisks (*, $P < 0.01$ and **, $P < 0.001$), and when still significant with Bonferroni correction ($P < 0.001$) values are in bold.

Factor	Dependant variables	
	Length	Weight
Initial length	**0.000****	0.191
Initial weight	0.327	**0.000****
Sex	0.931	0.001*
Clipped	0.897	0.865
Time	0.532	0.911
Sex + clipped	0.096	0.637
Clipped + time	0.423	0.888
Sex + time	0.510	0.952
Sex + clipped + time	0.184	0.702

Long-term effects

Both initial length and weight of individuals, as may be expected, significantly affected subsequent TL and weight. Sex also had a significant effect on the final weight of a seahorse. However, there was no significant difference between the growth rates in control and fin-clipped seahorses or over the duration of the experiment (22 weeks). When factors were combined, none were significant (Table 2). Figure 2 represents the average change in TL and weight over time for males and females, both clipped and control, there being no obvious trends as reflected by the non-significant results from the GLM which both had good fit ($R^2 > 75\%$). The small fluctuations in seahorse TL and weight were mostly likely caused by experimental error as the overall means were similar. Large individual fluctuations of two individuals were likely to be a result of mating and male pregnancy, however, this theory was untested as it was not possible to record the identity of females with hydrating eggs or pregnant males.

In total there were four mortalities during the experiment, all over a period of nine days starting on day 54 after dorsal fin clipping (week 16 of trial). Three further mortalities (two euthanized) occurred on days 56, 57 and 64 (week 17 and 18 of trial). The last seahorse that died had earlier been treated for gas bubble disease and was euthanized (day 64). After post-mortem examination by resident zoo vets two seahorses were diagnosed with mycobacteriosis as the probable cause of death (unpublished data). The cause of death of the remaining two seahorses was unknown. Mortality was divided equally between males and females, and between control and clipped seahorses.

The isolated DNA was visible as bands on a 1% agarose, ethidium bromide stained gel. The DNA isolated was sufficient to use in 20+ PCRs (Hall & Nawrocki, 1995).

DISCUSSION

This experiment found no significant short-term or long-term consequences of partial fin-clipping for seahorses. Dorsal fin

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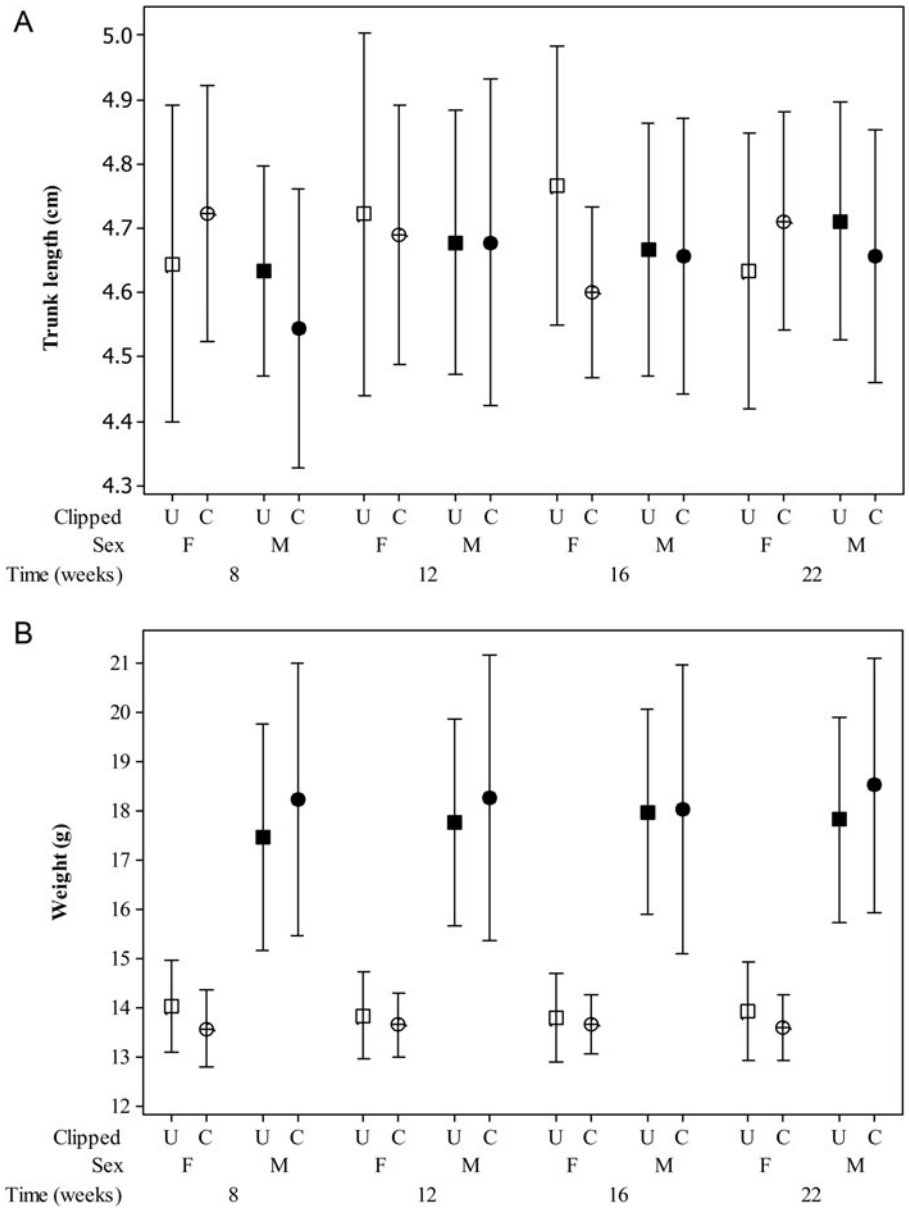

Fig. 2. Change in the mean trunk length (A) and mean weight (B) of seahorses during the course of the experimental trial. Unfilled, female; filled, male; square, unclipped; circle, clipped; U, unclipped; C, clipped; F, female; M, male. Bar shows 95% confidence interval, 206 × 137 mm (71 × 71 DPI).

re-growth was complete in four weeks. Long-term, partial fin-clipping had no significant effect on mortality, disease prevalence, or growth rates (weight or TL).

The current experiment showed total fin re-growth of partial clipped fins in *Hippocampus kuda* after 4 weeks, and agreed with previous anecdotal observations on this species (Lourie, 2003). Regrowth times were shorter than those for *Hippocampus guttulatus* and *Hippocampus hippocampus* that were observed *in situ* (6–8 weeks) (unpublished data). These seahorses were partially fin-clipped to sample tissue for a subsequent study. The present study showed complete re-growth of partially clipped seahorse fins with no obvious deformities in the regenerated fin. The higher ambient water temperatures inhabited by *H. kuda* may result in a higher metabolism in this species, and so explain faster regeneration. Both this experiment and previous anecdotal observations (Lourie, 2003) on *H. kuda* fin re-growth were made in captivity, whereas *H. guttulatus* observations were made *in situ.* Fish living *ex situ* generally experience more stable conditions and more regular food than they would in the wild, therefore we may expect tissue re-growth to be quicker for fish in captivity. In addition, there are no predators in an aquarium environment therefore some detrimental effects of the fin-clipping may not be detected. Seahorses are weak swimmers (Blake, 1976), with only a few small fins, partial fin-clipping a seahorse may have a greater effect on their swimming ability and manoeuvrability than in other fish species, impacting its ability to feed and to evade predation. However significant differences between fin re-growth times between this *ex situ* study and the *in situ* observations were not seen.

Studies of other fish species (Diekes *et al.*, 1999; & Katano & Uchida, 2006) all reported longer growth times (9–16 weeks). Although these studies report data on partial fin-

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clipping of a number of fins (caudal, dorsal, pelvic and anal), no detail on the size of the fin-clip is given. Other studies, that did not set out to explicitly test the effect of fin-clipping mention that fin re-growth had not occurred after many months (Coble, 1967, 1971).

Seahorses are notoriously problematic to keep in captivity (Koldewey & Martin-Smith, 2010), but increased susceptibility to disease may result from fin-clipping, which exposes damaged tissue to pathogens such as mycobacteria to which seahorses are particularly vulnerable. Fin-clipping cuts the fin rays and skin, but because the fin is very thin (21–25 μm) (Consi *et al.*, 2001), only a small surface area is exposed. The potential for infection should be noted, however, this trial suggests the added risk of infection through the damaged skin is low.

This study showed no significant change in either TL or weight of individuals over the duration of the experiment. However the fish used were mature adults and so no significant change in TL or weight was expected, other than that associated with reproduction. Small fluctuations in TL and weight observed in some individuals were probably due to experimental error in measurements of live fish. Results suggested that partial fin-clipping did not cause any significantly negative effect on mortality or growth of seahorses, however, four mortalities (two in clipped and two in control fish) did occur during the trial. There was no significant bias in mortality rate towards clipped individuals, suggesting that the deaths were not a result of partial fin-clipping. These results concur with those from previous studies that had tested partial fin-clipping on other fish species (Gjerde & Refstie, 1985; Pratt & Fox, 2002; Thompson *et al.*, 2005; Johnsen & Ugedal, 2008).

This study shows that partial fin clipping does not cause significant changes to either the mortality or growth of *H. kuda* in captivity. These results suggest that this technique of partial fin-clipping can be recommended as a viable method when collecting tissue from seahorses for DNA analysis. It would be interesting to carry out further investigation of this technique on seahorses *in situ* to confirm that results for wild fish agree with those for well-fed fish under controlled aquarium conditions.

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Correspondence should be addressed to:
L.C. Woodall
Zoology Department
The Natural History Museum
Cromwell Road
London, SW7 5BD
email: l.woodall@nhm.ac.uk

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