Skin pathology in Hawaiian goldring surgeonfish, *Ctenochaetus strigosus* (Bennett)

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Abstract

Twenty-eight goldring surgeonfish, *Ctenochaetus strigosus* (Bennett), manifesting skin lesions and originating from the north-western and main Hawaiian Islands were examined. Skin lesions were amorphous and ranged from simple dark or light discolouration to multicoloured tan to white sessile masses with an undulant surface. Skin lesions covered 2–66% of the fish surface, and there was no predilection for lesions affecting a particular part of the fish. Males appeared over-represented. Microscopy revealed the skin lesions to be hyperplasia, melanophoromas or iridophoromas. The presence of skin tumours in a relatively unspoiled area of Hawaii is intriguing. Explaining their distribution, cause and impact on survivorship of fish all merit further study because *C. strigosus* is an economically important fish in the region.

Keywords: chromatophoroma, goldring surgeonfish, histology, iridophoroma, melanophoroma, neoplasia.

Introduction

The health of organisms comprising aquatic ecosystems directly reflects the health of the ecosystem as a whole. In freshwater streams and lakes, the monitoring of fish health, particularly liver tumours (Baumann & Harshbarger 1995), has played a prominent role in aiding assessment of land-based pollution (Baumann 1992). Surveillance of liver tumours in wild fish has also proved useful to document the effects of land-based pollution in temperate marine ecosystems (Reynolds *et al.* 2003).

In contrast to temperate marine and freshwater ecosystems, relatively little information exists on tumours of fish in tropical marine ecosystems. Most studies on health of wild reef fish have focused on infectious or inflammatory diseases (Panek 2005). To date, the best documented tumour in tropical reef fish is neurofibromatosis of bicolor damselfish, *Pomacentrus partitus* Poey, that appears to be associated with a virus (Schmale 1991; Schmale, Gibbs & Campbell 2002). Other examples include chromatophoromas of unknown aetiology in butterflyfish, *Chaetodon multicinctus* (Garrett) and *C. miliaris* Quoy & Gaimard from Hawaii (Okihiro 1988), and, more recently, melanomas of coral trout, *Plectropomus leopardus* (Lacépède), in Australia (Sweet *et al.* 2012).

Goldring surgeonfish, *Ctenochaetus strigosus* (Bennett), also known as Kole in Hawaiian, are economically important fish often collected for the aquarium trade (Tissot & Hallacher 2003). During routine coral reef surveys in the Hawaiian Islands, we encountered skin lesions in this species that appeared as obvious discoloured areas. Our objectives were to systematically describe skin lesions in *C. strigosus* at the gross and microscopic level.

Materials and methods

Complete necropsies comprising external and internal examination were performed on 27 and one *C. strigosus* from the north-western and main Hawaiian Islands in 2005 and 2006, respectively. Twenty-one fish were measured (fork length, 0.1 cm) using calipers. Representative tissues including gastrointestinal tract, liver, spleen, cranial

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and caudal kidney, brain, heart, gill, swim bladder, skeletal muscle and skin (including lesions) were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 μm, stained with haematoxylin and eosin, and examined microscopically. On microscopy, skin lesions were characterized as hyperplasia (increased numbers of cuboidal epidermal cells with normal nuclear morphology) or neoplasia (locally invasive masses with microscopic evidence of disorganized cellular proliferation, anaplasia, dysplasia, nuclear polymorphism, disorganized cellular structure or atypia) (Groff 2004). Neoplasias were further classified as melanophoromas or iridophoromas. On microscopy, melanophoromas consisted of cells replete with intracytoplasmic melanin, whereas iridophoromas contained intracytoplasmic reflecting platelets (Bagnara & Matsumoto 2006). Fontana Masson stain was used to confirm the presence of melanin (Prophet *et al.* 1992).

Photogrammetry was performed on 18 of the fish for which we had available standardized photos to quantify the extent of gross lesions. Standard photographs (left and right side with cm scale bar) were taken of each fish, and the surface area (cm^2) occupied by skin lesion was measured using Image J (Schneider, Rasband & Eliceiri 2012). For purposes of analysis, each fish was compartmentalized as bilateral cranial (area rostral to caudal margin of operculum), bilateral caudal (area caudal to cranial insert of lateral scalpel), bilateral middle (remaining area), bilateral dorsal and ventral to lateral scalpel (Fig. 1a), and left and right side (all compartments). Per cent of total fish surface occupied by lesions in each compartment was then calculated as was per cent occupying left or right side. Mean per cent lesion cover of left and right or dorsal vs. ventral was compared with *t*-test, whilst analysis of variance was used to compare bilateral (left and right) per cent lesion cover for rostral, middle and caudal. Chi-square test was used to assess relationship between rostral, middle and caudal distribution vs. left and right side and dorsal/ventral vs. left and right side. Level of significance for all analyses was 0.05.

Results

Gross lesions manifested as distinct amorphous dark brown, tan or white mottled areas (Fig. 1b–d). In three cases, lesions were raised or undulant and occasionally manifested ulceration and haemorrhage (Figs 1c–d). On histology, putative early lesions consisted of hyperplastic epidermal cells (Fig. 2b). Putative early neoplastic lesions consisted of papillomatous growths comprised of haphazard bundles of fusiform cells mixed with melanophores (Fig. 2c). In more advanced lesions, trabeculae of haphazardly arranged fusiform cells infiltrated underlying dermis and were interspersed with clumps of melanophores (Fig. 2d–f) that stained positive for melanin with Fontana Masson (Fig. 1e). In gross lesions where masses were white (Fig. 1d), histology revealed haphazardly arranged bundles of fusiform cells mixed with clusters of

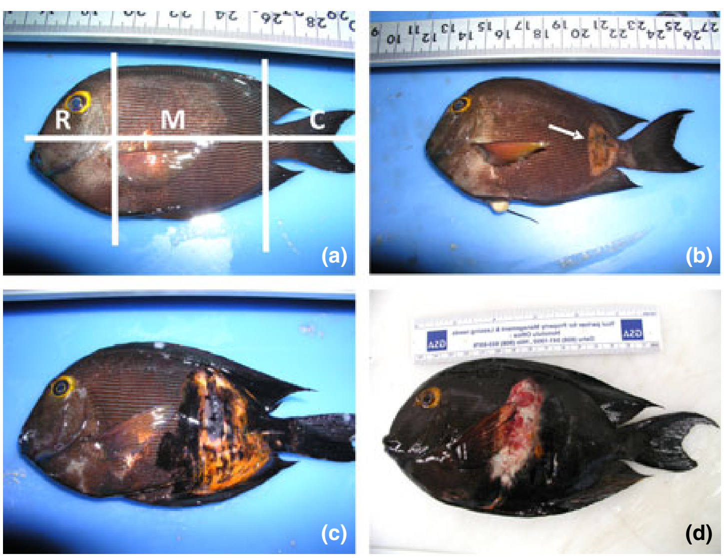

Figure 1 *Ctenochaetus strigosus.* (a) Normal fish; note generally uniform pigmentation. Vertical lines delineate rostral (R), middle (M) and caudal (C) compartments, whilst horizontal lines delineate dorsal (top) and ventral (bottom). (b) Localized discolouration at base of tail (arrow); epidermal hyperplasia on microscopy. (c) Diffuse mottled black to orange raised undulant area; melanophoroma on microscopy. (d) Elliptical raised rugose white mass with ulceration and haemorrhage; iridophoroma on microscopy.

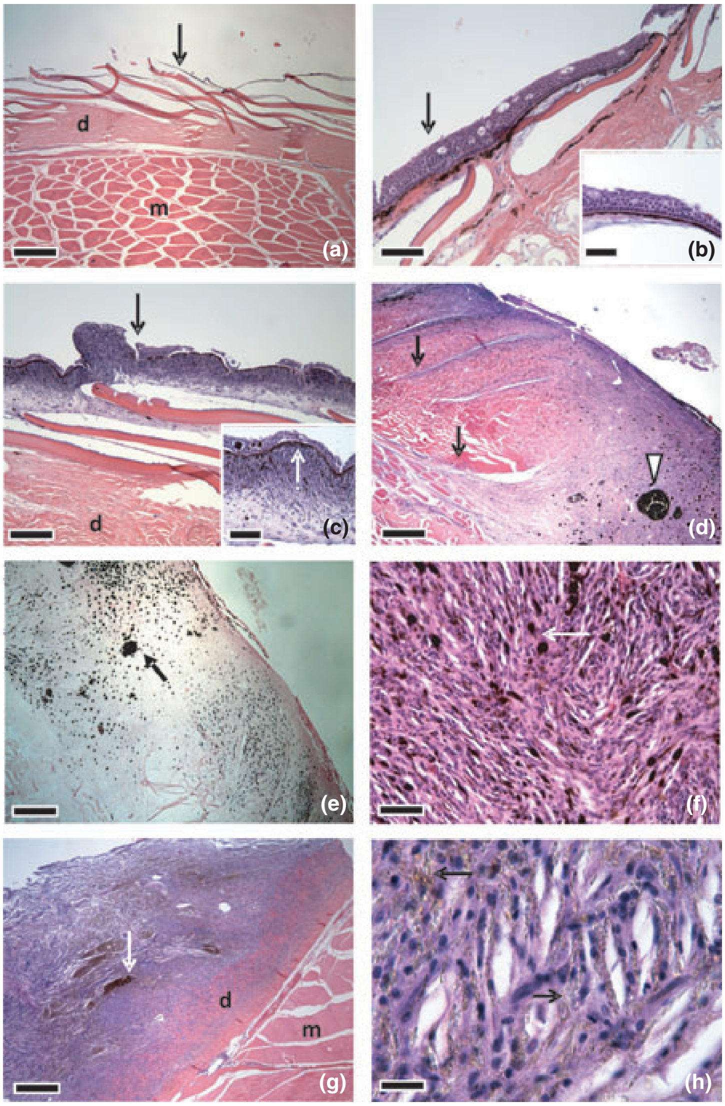

Figure 2 *Ctenochaetus strigosus* normal skin (a), hyperplasia (b), melanophoroma (c–f) and iridophoroma (g–h). (a) Normal skin; note thin layer of epidermis on scales (arrow), dermis (d) and skeletal muscle (m). Bar = 200 μm. (b) Hyperplasia. Note multiple layers of well-organized cuboidal cells with uniform morphology and nuclei interspersed with mucus cells (vacuoles). Bar = 30 μm. Inset shows higher magnification; bar = 40 μm. (c) Putative early stage melanoma. Note bundles of fusiform basophilic cells forming papillomatous growths among a loose connective tissue stroma that surrounds and partially absorbs underlying scales. Bar = 20 μm. Inset. Note haphazard sheets of cells with subsurface layer of melanocytes (white arrow), bar = 40 μm. (d) Melanophoroma. Note bundles of basophilic cells arranged in whorls with trabeculae infiltrating dermis (black arrow) with islands of melanocytes (white arrowhead) interspersed throughout. Bar = 200 μm. (e) Fontana Masson stain of D. Note clusters of melanocytes (arrow). Bar = 200 μm. (f) Melanophoroma; note bundles of spindle-shaped cells mixed with melanocytes (arrow). Bar = 20 μm. (g) Iridophoroma; note bundle of fusiform cells effacing epidermis and infiltrating dermis with clusters of golden-brown iridophores (white arrow); contrast with D. Bar = 200 μm. (h) Iridophoroma; note bundles of fusiform cells with intracytoplasmic clumps of golden-brown reflecting platelets birefringent in polarized light (arrows) (iridophores) interspersed with lanceolate clefts. Bar = 6 μm. Abbreviations: d-dermis, m-skeletal muscle.

iridophores (Fig. 2g) replete with intracytoplasmic reflecting platelets birefringent in polarized light (Fig. 2h). Masses were vascularized, invaded dermis but not skeletal muscle and were occasionally ulcerated or haemorrhagic. Of 28 fish with gross skin lesions, 12 manifested hyperplasia, eight neoplasia, one diffuse ulceration and seven had no microscopic lesions. Of the three fish with

Table 1 Percent coverage of body by skin lesions for 18 *Ctenochaetus strigosus* partitioned by side (left or right) or region (rostral, mid, or caudal) or dorsal and ventral

	Mean	SD	Range	*n*
Overall %	21	15	2–66	18
Right %	20	15	2–59	12
Left %	22	18	2–72	17
Rostral %	9	6	2–14	3
Mid %	14	12	1–40	13
Caudal %	13	10	1–36	12
Dorsal %	11	9	3–34	18
Ventral %	10	8	0–36	18

lesions having raised or undulant surface, all had microscopic evidence of neoplasia.

Skin lesions covered 1–72% of fish surfaces and affected mainly the left side, mid and caudal portions with equal dorsal and ventral distributions (Table 1). Fish ranged from 4.9 to 22.3 cm, 13 ± 3.6 (mean ± SD). Of seven fish with neoplasia that were measured, range of fork lengths was 7–22.3 cm (14.7 ± 4.7). Eleven of 18 fish quantified for lesion coverage had lesions bilaterally. In six cases, lesions were present only on the left side of the fish, whereas unilateral lesions on the right were seen in one fish only. For cranial to caudal distribution, five fish each had lesions in the caudal, middle or caudal and middle compartment, two had lesions in the cranial and middle compartment, and one had lesions in all three compartments. Dorsal and ventral lesions were equally distributed. No significant difference was seen in mean per cent lesion cover between left and right side, rostral, middle and caudal, or dorsal and ventral. No significant association was seen between left and right side vs. rostal, middle and caudal or left and right vs. dorsal and ventral. Of 28 fish with skin lesions, 17 were males, seven females and the remainder unknown.

Discussion

We found the distinct discoloured areas (skin lesions) in *C. strigosus* to be chromatophoromas making this the third species of Hawaiian reef fish where this disease has been documented with chromatophoromas previously found in two species of butterflyfish (Okihiro 1988). Microscopically, tumours in *C. strigosus* were predominantly melanophoromas with iridophoromas uncommon. We saw localized invasiveness of tumour tissues into dermis but no metastases tending to confirm observations in butterflyfish (Okihiro 1988) and other fish (Groff 2004) that skin tumours in this group of animals are seldom metastatic. We suspect that the hyperplasia seen in many *C. strigosus* was an early manifestation of tumours. Studies of chromatophoromas in wild temperate marine fish have drawn similar conclusions (Kimura *et al.* 1984). Observations and biopsies of affected fish over time would help confirm this conjecture. Unlike butterflyfish where iridophoromas are more common (Okihiro 1988), melanophoromas appear more common in goldring surgeonfish, a phenomenon more akin to that seen in temperate marine fish such as rock fish, *Sebastes* spp., from California (Okihiro *et al.* 1992) and the North Atlantic (Bogovski & Bakai 1989) and croakers*, Nibea mitsukurii* (Jordan & Snyder), from Japan (Kimura *et al.* 1984). Although not significant, there appeared to be a predilection of skin lesions on the left side. This along with the preponderance of males affected merits further investigations. Female *Sebastes mentella* (Travin) from the Atlantic were more often affected by chromatoblastomas (Bogovski & Bakai 1989), whereas melanophoromas were more common in male *Sebastes flavidus* Ayres and *Sebastes goodei* (Eigenmann & Eigenmann) with equal frequency in males and females for *Sebastes paucispinis* Ayres (Okihiro *et al.* 1992). The only iridophoroma in surgeonfish was observed in the single sample from the main Hawaiian Islands (Maui); whether this tumour type is more common in the main Hawaiian Islands awaits further studies. Based on this sample size, it appears small to large size classes are susceptible to neoplasia, so senescence as a potential cause of this disease is unlikely.

In Hawaiian butterflyfish, prevalence of chromatophoromas exceeded 20% in some areas, and it was postulated that runoff from sugar cane plantations may have played a role in the genesis of tumours (Okihiro 1988). In contrast, all but one of the affected surgeonfish in this study came from the north-western Hawaiian Islands where few anthropogenic stressors exist, so we think it unlikely that agricultural contaminants play a role. However, systematic surveys of populations of *C. strigosus* in the main vs. north-western Hawaiian Islands are needed to examine spatial patterns of disease occurrence, thereby allowing more robust formulation of hypotheses concerning potential contributing environmental factors.

We have not yet conducted studies to determine the cause of skin cancer in *C. strigosus*; however, skin tumours in other fish species have multiple potential causes including environmental contaminants (Black & Baumann 1991), viruses (Anders & Yoshimizu 1994), UV light (Gimenez-Conti *et al.* 1999) and genetic predisposition (Kimura *et al.* 1984). Okihiro (1988) failed to detect viral-like particles in Hawaiian butterflyfish tumours on electron microscopy; however, only ca. 50% of virus-induced skin tumours in fish have evidence of viruses on ultrastructure (Anders & Yoshimizu 1994), so this is not a reliable indicator of virus-induced tumours in fish. Certain marine toxins like okadaic acid produced by dinoflagellates can induce tumours in animals experimentally (Suganuma *et al.* 1988). *C. strigosus* are primarily detritivores and herbivores (Robertson & Gaines 1986) so they could be exposed to sediment-borne toxicants (Harshbarger & Clark 1990) or tumour-inducing marine toxins from dinoflagellates. We have no evidence that UV light plays a role in genesis of skin tumours in surgeonfish.

Future research should focus on ruling out infectious aetiologies for tumours in Hawaiian reef fish, by inoculation of experimental subjects with cell-free tumour extracts (Schmale 1995), electron microscopy or cell culture in attempts to isolate viruses (Schmale, Aman & Gill 1996). Identification of areas with high tumour prevalence in the main or north-western Hawaiian Islands with appropriate low prevalence controls may also set the stage for sorting out the role of environmental factors in the genesis of tumours in these species (Baumann 1992). Care should be taken not to diagnose skin lesions in surgeonfish as neoplasms based on gross observations alone unless these lesions are protuberant; in fact, only 28% of skin lesions were confirmed as neoplasia histologically. Finally, longer-term systematic surveys at representative index sites could shed more light on temporal patterns of tumour occurrence indicating whether these tumours are increasing or decreasing over time. Similar surveys have been used successfully to document the decline of tumour disease in other marine organisms in Hawaii such as sea turtles (Chaloupka, Balazs & Work 2009).

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References

Anders K. & Yoshimizu M. (1994) Role of viruses in the induction of skin tumours and tumour-like proliferations of fish. *Diseases of Aquatic Organisms* **19**, 215–232.

Bagnara J.T. & Matsumoto J. (2006) Comparative anatomy and physiology of pigment cells and nonmammalian tissues. In: *The Pigmentary System: Physiology and Pathophysiology* (ed. by J.J. Norlund, R.E. Boisy, V.J. Hearing, R.A. King, W.S. Oetting & J. Orton), pp. 1–59. Blackwell, Hoboken, USA.

Baumann P.C. (1992) The use of tumors in wild populations of fish to assess ecosystem health. *Journal of Aquatic Ecosystem Health* **1**, 135–146.

Baumann P.C. & Harshbarger J.C. (1995) Decline in liver neoplasms in wild brown bullhead catfish after coking plant closes and environmental PAHs plummet. *Environmental Health Perspectives* **103**, 168–170.

Black J.J. & Baumann P.C. (1991) Carcinogens and cancers in freshwater fishes. *Environmental Health Perspectives* **90**, 27–33.

Bogovski S.P. & Bakai Y.I. (1989) Chromatoblastomas and related pigmented lesions in deepwater redfish, *Sebastes mentella* (Travin), from North Atlantic areas, especially the Irminger Sea. *Journal of Fish Diseases* **12**, 1–13.

Chaloupka M., Balazs G.H. & Work T.M. (2009) Rise and fall over 26 years of a marine epizootic in Hawaiian green sea turtles. *Journal of Wildlife Diseases* **45**, 1138–1142.

Gimenez-Conti I., Woodhead A.D., Harshbarger J.C., Kazianis S., Setlow R.B. & Nairn R. (1999) A proposed classification scheme for *Xiphophorus* melanomas based on histopathologic analyses. *Marine Biotechnology* **3**, S100–S106.

Groff J.M. (2004) Neoplasia in fishes. *Veterinary Clinics of North America, Exotic Animal Practice* **4**, 705–756.

Harshbarger J.C. & Clark J.B. (1990) Epizootiology of neoplasms in bony fish of North America. *Science of the Total Environment* **94**, 1–32.

Kimura I., Taniguchi N., Kumai H., Tomita I., Kinae N., Yoshizaki K., Ito M. & Ishikawa T. (1984) Correlation of epizootiological observations with experimental data: chemical induction of chromatophoromas in the croaker, *Nibea mitsukurii*. *National Cancer Institute Monograph* **65**, 139–154.

Okihiro M.S. (1988) Chromatophoromas in two species of Hawaiian butterflyfish, *Chaetodon multicinctus* and *C. miliaris*. *Veterinary Pathology* **25**, 422–431.

Okihiro M.S., Whipple J.A., Groff J.M. & Hinton D.E. (1992) Chromatophoromas and related hyperplastic lesions in Pacific rockfish (*Sebastes* spp.). *Marine Environmental Research* **34**, 53–57.

Panek F. (2005) Epizootics and disease of coral reef fish in the tropical western Atlantic and Gulf of Mexico. *Reviews in Fisheries Science* **13**, 1–21.

Prophet E.B., Mills B., Arrington J.B. & Sobin L.H. (1992) *Laboratory Methods in Histotechnology*. Armed Forces Institute of Pathology, Washington.

Reynolds W.J., Feist S.W., Jones G.J., Lyons B.P., Sheahan D.A. & Stentiford G.D. (2003) Comparison of biomarker and pathological responses in flounder (*Platichthys flesus L.*) induced by ingested polycyclic aromatic hydrocarbons (PAH) contamination. *Chemosphere* **52**, 1135–1145.

Robertson R.R. & Gaines S.D. (1986) Interference competition structures habitat use in a local assemblage of coral reef surgeonfishes. *Ecology* **67**, 1372–1383.

Schmale M.C. (1991) Prevalence and distribution patterns of tumors in bicolor damselfish (*Pomacentrus partitus*) on South Florida reefs. *Marine Biology* **109**, 203–212.

Schmale M. (1995) Experimental induction of neurofibromatosis in bicolor damselfish. *Diseases of Aquatic Organisms* **23**, 201–212.

Schmale M.C., Aman M.R. & Gill K.A. (1996) A retrovirus isolated from cell lines derived from neurofibromas in bicolor damselfish (*P. partitus*). *Journal of General Virology* **77**, 1181–1187.

Schmale M., Gibbs P. & Campbell C. (2002) A virus-like agent associated with neurofibromatosis in damselfish. *Diseases of Aquatic Organisms* **49**, 107–115.

Schneider C.A., Rasband W.S. & Eliceiri K.W. (2012) NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* **9**, 671–675.

Suganuma M., Fujiki H., Suguri H., Yoshizawa S., Hirota M., Nakayasu M., Ojika M., Wakamatsu K., Yamada K. & Sugimura T. (1988) Okadaic acid: an additional non-phorbol-12-tetradecanoate-13-acetate-type tumor promoter. *Proceedings of the National Academy of Sciences* **85**, 1768–1771.

Sweet M., Kirkham N., Bendall M., Currey L., Bythell J. & Heupel M. (2012) Evidence of melanoma in wild marine fish populations. *PLOSOne* **7**, e41989.

Tissot B.N. & Hallacher L.E. (2003) Effects of aquarium collectors on coral reef fishes in Kona, Hawaii. *Conservation Biology* **17**, 1759–1768.

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