เชียงใหม่สัตวแพทยสาร 2560; 15(2): 109-115. DOI: 10.14456/cmvj.2017.10

เชียงใหม่สัตวแพทยสาร

Chiang Mai Veterinary Journal

ISSN; 1685-9502 (print) 2465-4604 (online)

Website; www.vet.cmu.ac.th/cmvj

Original Article

Brain histopathology and optic lobe atrophy of Short mackerel (*Rastrelliger brachysoma*) in the Upper Gulf of Thailand

Sinlapachai Senarat[1], Wannee Jiraungkoorskul[2], Theerakamol Pengsakul[3], F. Gerald Plumley[1], Pisit Poolprasert[4], Jes Kettratad[1,*]

[1]*Department of Marine Science, Faculty of Science, Chulalongkorn University, Bangkok 10330*

[2]*Department of Pathobiology, Faculty of Science, Mahidol University, Bangkok 10400*

[3]*Faculty of Medical Technology, Prince of Songkla University, Songkhla, 90110*

[4]*Program of Biology, Faculty of Science and Technology, Pibulsongkram Rajabhat University, Mueang, Phitsanulok, 65000*

Abstract Short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851), is an economically important marine fish in the Gulf of Thailand that is potentially threatened by anthropogenic factors. Fifty specimens were obtained between October 2013 to February 2014 from wild populations, processed using standard histological techniques and stained with Harris's hematoxylin and eosin. The results revealed important histopathological findings in the brain tissues of *R. brachysoma* including optic lobe atrophy with neuronal degeneration, vacuolar degeneration, melanomacrophage centers and cerebellar neuronal cell degeneration (12% prevalence). This preliminary study provided evidence that *R. brachysoma* in the Upper Gulf of Thailand lives under stressful conditions that likely impact brain function and possibly the fish's overall health.

Keywords: histology, histopathology, neuronal cell, short mackerel, Thailand

* **Corresponding author:** Jes Kettratad, Department of Marine Science, Faculty of Science, Chulalongkorn University Bangkok 10330

E-mail: kettratadjes@gmail.com

Article history; *received manuscript: 7 June 2017, accepted manuscript: 14 July 2017, published online: 20 July 2017*

บทความต้นฉบับ

จุลกายพยาธิวิทยาของสมองและการฝ่อของออพติก โลบ ปลาทู *(Rastrelliger brachysoma)* ในบริเวณอ่าวไทยตอนบน

ศิลปชัย เสนารัตน์[1] **วรรณีย์ จิรอังกูรสกุล**[2] **ธีรกมล เพ็งสกุล**[3] F. Gerald Plumley[1]
พิสิษฐ์ พูลประเสริฐ[4] **เจษฎ์ เกษตระทัต**[1,*]

[1]*ภาควิชาวิทยาศาสตร์ทางทะเล คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพ 10330*
[2]*ภาควิชาพยาธิชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยมหิดล กรุงเทพฯ 10400*
[3]*คณะเทคนิคการแพทย์ มหาวิทยาลัยสงขลานครินทร์ อำเภอหาดใหญ่ สงขลา 90110*
[4]*สาขาชีววิทยา คณะวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยราชภัฏพิบูลสงคราม อำเภอเมือง พิษณุโลก 65000*

บทคัดย่อ ปลาทู *Rastrelliger brachysoma* จัดเป็นปลาทะเลเศรษฐกิจที่สำคัญในอ่าวไทยที่อาจถูกคุกคามโดยตัวแปรที่เกิดขึ้นจากกิจกรรมมนุษย์ (anthropogenic factors) ตัวอย่างปลาทูทั้งหมดจำนวน 50 ตัวถูกเก็บระหว่างเดือนตุลาคม พ.ศ.2556 ถึงเดือนกุมภาพันธ์ พ.ศ.2557 นำมาผ่านกระบวนการเตรียมตัวอย่างมาตรฐานทางมิญชวิทยา (standard histological techniques) และย้อมสีด้วย hematoxylin และ eosin (H&E) ผลการศึกษาพบจุลกายพยาธิของสมองปลาทูที่สำคัญคือ การฝ่อของสมองที่ประกอบด้วยการฝ่อของออบติก โลบ (optic lobe) ร่วมกับการเสื่อมของเนื้อเยื่อประสาท การเสื่อมแบบแวคูโอลา (vacuolar degeneration) การสะสมของเมลาโนแมคโครฟาจ (melanomacrophage centers) และการเสื่อมของเซลล์ประสาทในซีรีเบลลัม (ความชุกร้อยละ 12) การศึกษาเบื้องต้นครั้งนี้ทำให้ทราบว่าปลาทูในบริเวณอ่าวไทยตอนบนที่อยู่ในสภาวะความเครียดมีผลกระทบต่อหน้าที่ของสมองและปัญหาสุขภาวะโดยรวม

คำสำคัญ มิญชวิทยา จุลกายพยาธิวิทยา เซลล์ประสาท ปลาทู ประเทศไทย

* **ผู้รับผิดชอบบทความ** เจษฎ์ เกษตระทัต ภาควิชาวิทยาศาสตร์ทางทะเล คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย ปทุมวัน กรุงเทพฯ 10330
อีเมล์: kettratadjes@gmail.com

ข้อมูลบทความ *วันที่ได้รับบทความ 7 มิถุนายน พ.ศ.2560 วันที่ได้รับการตีพิมพ์ 14 กรกฎาคม พ.ศ.2560 วันที่ตีพิมพ์ออนไลน์ 20 กรกฎาคม พ.ศ.2560*

Introduction

Previous reports showed that the Upper Gulf of Thailand is an economically important region (Sutthakorn, 1998; Sritakon et al., 2006). It is a regionally important bay that receives water from hundreds of small, medium, and large cities and towns, and from numerous fisheries-related activities as well as from aquacultural and industrial areas; four of the largest rivers in Thailand empty into the upper gulf, including the Chaopaya, Tha-Chin, Bangprakong, and Maeklong Rivers. According to previous studies, a wide variety of pollutants, especially heavy metals (e.g., waters enriched with cadmium, iron, mercury and lead) and petroleum hydrocarbons have contaminated the sediment and water for a long time (Hungspreugs and Yuangthong, 1983; Cheevaporn and Menasveta, 2003; Wattayakorn, 2012). High pollution levels in the Upper Gulf of Thailand have potentially serious negative impacts on the entire ecosystem as the health of aquatic organisms in the region are adversely impacted.

Our previous research on the health status of *Rastrelliger brachysona*, an economically important marine fish of the Upper Gulf of Thailand, utilized histopathological biomarker to reveal pathologies in several organs (Hinton et al., 2001; Dietrich and Krieger, 2009; Senarat et al., 2015). Biomarkers are extremely useful tools that are generally used to indicate environmental problems in both sub-lethal and chronic effects (Fatma, 2009; Nikalje et al., 2012). One of the most interesting lesions observed in earlier work (Snearat et al, 2015) was brain atrophy, which was observed in field populations of *R. brachysoma.* These brain lesions require further clarification. In this study, brain histopathological features and brain atrophy of *R. brachysoma* were described in greater detail. Insights from this work provide a better understanding of brain lesions in this fish, as well as deeper insights into the health of this fish and, by extension, the local ecosystem.

Materials and Method

Adult *R. brachysoma* (15 to 18 cm standard length, n=50) were captured by bamboo strake traps from Samut Songkram Province on the Upper Gulf of Thailand (13°16'18.4"N, 100°02'13.4"E), during October 2013 to February 2014. The experimental protocol was approved by the Animal Care and Use Committee of the Faculty of Science in accordance with the guide for the care and use of laboratory animals prepared by Chulalongkorn University (Protocol Review No. 1423003). All samples were euthanized by rapidly cooling shock (Wilson *et al*., 2009) and brains were surgically removed. All samples were fixed in Davidson's fixative (about 48 h at room temperature) and processed using standard histological techniques (Bancroft and Gamble, 2002). The brains were sectioned with 5 µm thickness and stained with Harris's hematoxylin and eosin (H&E) (Bancroft and Gamble, 2002). Histopathological alterations of brain and whole-brain atrophy sections were observed under the

light microscope and data recorded as a mean prevalence.

Results and Discussion

Our previous observation included details on the brain of *R. brachysoma* in both gross anatomy and histology (Senarat et al., 2016). This earlier work provided baseline data for the current research, which included fish with several prominent brain lesions (Figures 1-2). Anatomical and histopathological micrographs of the brain of *R. brachysoma* revealed prominent lesions associated with brain atrophy. Optic lobe atrophy in both hemispheres was anatomically diagnosed (4% prevalence, n=2 of 50) (Figures 1A-1B). Histological analysis of the optic lobe revealed prominent neuronal degeneration in the stratum album and griseum (Figures 2A-1B). The results presented in this study reveal the previously undocumented incidence of optic lobe atrophy in *R. brachysoma*. It is noteworthy that the lesion might be related to loss of function/ dysfunction of the optic lobe, which will be further studied.

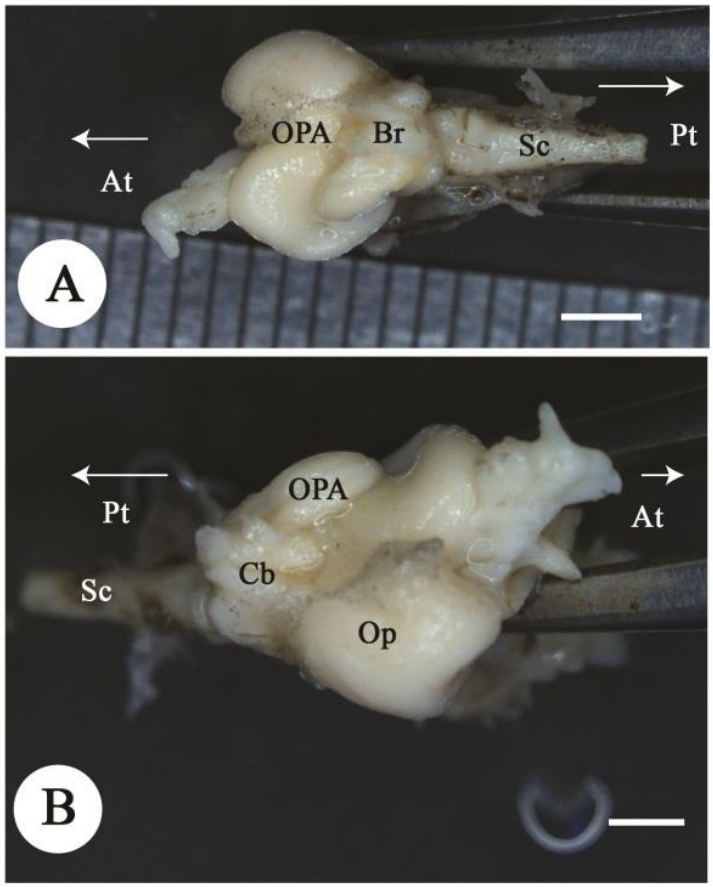

Figure 1. Morphology of the brain (Br) and optic lobe atrophy (OPA) in *R. brachysoma* brain sections (A-B) Labels: anterior part (At), cerebellum (Cb), optic lobe (OP), posterior part (Pt), Spinal cord (Sc). Scale bar A, B=2 mm. (Note: Figure A. Left oblique view, Figure B. Right lateral view)

Prominent histopathological alterations of brain sections included vacuolar degeneration, melanomacrophage centers (MMC) and neuronal cell degeneration of the cerebellum (12% prevalence, n=6 of 50) (Figures 2D-2E). The appearance of vacuolar degeneration of the cerebellum also was observed in this study (Figure 1B). This could be due to the similar effects of a variety of different pollutants such as glyphosate and endosulfan (Sarma et al., 2009; No et al., 2014). Meyers and Hendricks (1985) suggested that vacuolar degeneration might indicate an imbalance in biochemical synthesis, inhibition of protein synthesis, energy depletion, desegregation of microtubules, and shifts in substance utilization. It is possible that the occurrence of MMC in *R. brachysoma* brain was a direct result of fish living under stressful conditions (e.g., overcrowding, excessive noise and aggression) (Alvarez-Pellitero et al., 2007; Sitja-Bobadilla, 2008) and/or poor water quality with contaminants (Patiño et al., 2003). Several investigators (Marty et al., 2003; Van Dyk et al., 2010) have suggested increased MMC in tissues is associated with infectious diseases through the change of environmental quality or stress responses to the exposure to toxicants. Our histological analysis of this lesion could provide a useful tool for monitoring the health status of wild *R. brachysoma* and serve as reference data and warning in regards to deterioration of the environment of the Upper Gulf of Thailand.

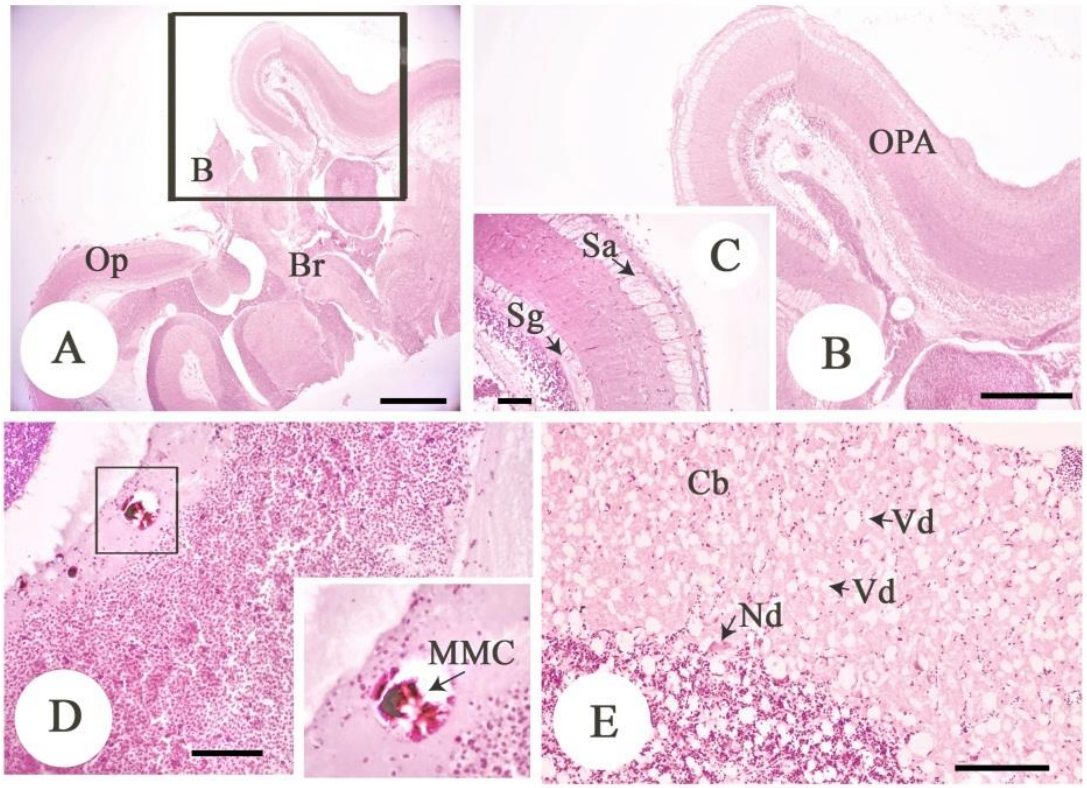

Figure 2. Light photomicrographs of histopathology in *R. brachysoma* brain sections (A-E) Labels: brain (Br), melanomacrophage centers accumulation (MMC), vacuolar degeneration (Vd), neuronal cell degeneration (Nd) of cerebellum (Cb), optic lobe (OP), optic lobe atrophy (OPA) neuronal degeneration in the stratum album (Sa) and griseum (Sg). Scale bar A, B, D, E=50 µm; C=20 µm.

Conclusion

This report has revealed brain histopathology, especially optic lobe atrophy, in *R. brachysoma* in the Upper Gulf of Thailand. It is tempting to extrapolate these results as an indication that this fish has lived under stressed conditions. Moreover, the brain pathologies observed here are associated with declining fish health related to physiology and/or behavior. This information may be applicable as an early warning for this particular fish in this particular marine ecosystem, but at a wider scale, could serve as a warning for fish health and the environmental damage at large scales.

Acknowledgement

This research was supported by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship (to S. Senarat).

References

Alvarez-Pellitero, P., Palenzuela O., Sitjà-Bobadilla A., 2007. Histopathology and cellular response in *Enteromyxum leei* (Myxozoa) infections of *Diplodus puntazzo* (Teleostei). Parasitol. Int. 57, 110 –120.

Bancroft, J.D., Gamble, M., 2002. Theory and Practice of Histological Techniques. Churchill Livingstone, Oxford.

Cheevaporn, V., Menasveta, P., 2003. Water pollution and habitat degradation in the Gulf of Thailand. Mar. Pollut. Bull. 47, 43 –51.

Dietrich, D.R., Krieger, H.O., 2009. Histological analysis of endocrine disruptive effects in small laboratory fish. John Wiley and Sons, New Jersey, U.S.A.

Fatma, A.S.M., 2009. Histopathological studies on *Tilapia zillii* and *Solea vulgaris* from Lake Qarun, Egypt. World J. Fish Mar. Sci. World. 1, 29–39

Hinton, D.E., Segner, H., Braunback, T. 2001. Toxic response of liver. In: Shlenk, D., Benson, W.H. (Eds.), Target organ toxicity in marine and fresh water teleosts, Taylor and Francis, London, UK, pp. 224–268.

Hungspreugs, M., Yuangthong, C., 1983. A history of metal pollution in the Upper Gulf of Thailand. Mar. Pollut. Bull. 14, 465 –469.

Meyers, T.R., Hendricks, J.D., 1985. Histopathology. In: Rand, G.M., Petrocelli, S.R. (Eds.), Fundamentals of aquatic toxicology, Hemisphere Publishing Corp, New York, U.S.A, pp 283 –331.

Marty, G.D., Hoffmann, A., Okihiro, M.S., Hepler, K., Hanes, D., 2003. Retrospective analyses: bile hydrocarbons and histopathology of demersal rockfish in Prince William sound, Alaska, after the Exxon Valdez oil spill. Mar. Environ. Res. 56, 569 –584.

No, E., Sa, E., Mo, A., EE, E., 2014. Histopathological changes in the brain tissue of Africa catfish expose to glyphosate herbicide. J. Appl. Sci. Environ. Manag. 18, 275–280.

Nikalje, S.B., Muley, D.V., Angadi, S.M., 2012. Histopathological changes in gills of a freshwater major carp, *Labeo rohita* after acute and chronic exposure to textile mill effluent (tme). Int. J. Environ. Sci. 3, 108 – 118.

Patiño, R., Wainscott, M.R., Cruz-Li, E.I., Balakrishnan, S., McMurry, C., Blazer, V.S., Anderson, T.A. 2003. Effects of ammonium perchlorate on the reproductive performance and thyroid follicle histology of zebra fish. Environ. Toxicol. Chem. 22, 1115–1121.

Sarma, D.J., Kenyon, L.C., Hingley, S.T., Shindler, K.S., 2009. Mechanism of primary axonal damage in a viral model of multiple sclerosis. J. Neurosci. 29, 10272–10280.

Senarat S., Kettratad J., Poolprasert P., Boonyoung P., Kangwanrangsan N., Jiraungkoorskul, W., 2015. Hepatic histopathology in *Rastrelliger brachysoma* (Bleeker, 1851) from The Upper Gulf of Thailand. APCEAS, Osaka, Japan. 25-27 August, 2015. pp. 369–374.

Senarat, S., Kettretad, J., Jiraungkoorskul, W., 2016. Neuroanatomy and histology of the central nervous system in short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) Walailak J. Sci. & Tech. 13, 531–541.

Sitja-Bobadilla, A., 2008. Fish immune response to myxozoan parasites. Parasitology. 15, 420–425.

Sritakon, T., Chotithammo, S., Vechprasit, S., 2006. Reproductive biology of short mackerel *Rastrelliger brachysoma* (Bleeker, 1851) and Indian mackerel *R. kanagurta* (Cuvier, 1817) in the Southern Gulf of Thailand. Department of Fisheries, Thailand.

Sutthakorn, P., 1998. Biological aspects of short mackerel, *Rastrelliger brachysoma* (Bleeker, 1851) of the Andaman Sea Coast of Thailand. Department of Fisheries, Thailand.

van Dyka, J.C., Marchanda, M.J., Smita, N.J., Pietersea, G.M., 2010. A histology-based fish health assessment of four commercially and ecologically important species from the Okavango Delta panhandle, Botswana. Afr. J. Aquat. Sci. 34, 273–282.

Wattayakorn, K., 2012. Petroleum pollution in the Gulf of Thailand: A historical review. Coast. Mar. Sci. 35, 234–245.

Wilson, J.M., Bunte, R.M., Carty, A.J., 2009. Evaluation of rapid cooling and tricaine methanesulfonate (MS222) as methods of euthanasia in zebrafish (*Danio rerio*). J. Am. Assoc. Lab. Anim. Sci. 48, 785–787.