LAPORAN AKHIR TAHUN
PENELITIAN BERBASIS KOMPETENSI
(PBK)

PERUBAHAN STRUKTUR, WARNA DAN FUNGSI MELANOFOR PADA SISIK IKAN NILA (*OREOCHROMIS NILOTICUS*) SETELAH DIPAPAR DENGAN KADMIUM: SEBAGAI BIOINDIKATOR POTENSIAL PENCEMARAN KADMIUM

TAHUN KE-1 DARI RENCANA 2 TAHUN

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SESUAI DENGAN PERJANJIAN PENDANAAN PENELITIAN DAN PENGABDIAN KEPADA MASYARAKAT
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HALAMAN PENGESAHAN

Judul	: Respon Fisiologis Pemaparan Tembaga (Cu) pada Ikan Nila (Oreochromis niloticus): Evaluasi terhadap Regulasi Ionik, Osmotik, dan Metallothionein dalam Insang
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RINGKASAN

Kadmium (Cd) merupakan bahan pecemar penting di lingkungan perairan. Penelitian kami sebelumnya menunjukkan bahwa Cd dapat terakumulasi pada insang dan mempengaruhi peningkatan kadar *metallothionein* serta merubah struktur insang ikan nila (*Oreochromis niloticus*) (Nursanti et al. 2017). Penelitian tentang pengaruh Cd terhadap insang sebagai organ osmoregulasi juga telah kami lakukan dan saat ini sedang di*submit* di jurnal Zoology dan Ecology (dengan status *under review*). Setelah mengetahui peran insang sebagai target pengaruh bahan pencemar khususnya Cd perlu dilakukan penelitian terhadap sisik ikan, mengingat sisik ikan terdapat di bagian luar tubuh dan secara kontinyu berhubungan langsung dengan bahan pencemar yang terdapat dalam air. Mengapa perlu melakukan penelitin ini? Alasan pentingnya dijelaskan dibawah ini.

Kulit ikan ditutup oleh sisik yang mempunyai peran penting melindungi tubuh dari abrasi air, gesekan dengan batu karang, kehilangan ion, dan invasi oleh pathogen. Selain itu perubahan warna sisik juga merupakan mekanisme adaptasi terhadap perubahan lingkungan.

Ikan nila (*Oreochromis niloticus*) tubuhnya ditutup oleh sisik sikloid. Sisik sikloid mempunyai sebuah *focus* dan terbagi ke dalam bagian anterior, posterior dan lateral. Bagian *focus* dikelilingi oleh sirkuli. Pada bagian posterior sisik terdapat sel yang berisi pigment (kromatofor). Ada dua tipe kromatofor: yang pertama merefleksikan cahaya (missal iridofor) dan yang kedua menyerap cahaya (seperti melanofor dan sianofor). Melanofor berisi pigmen warna hitam dan coklat. Perubahan warna sisik dapat berlangsung melalui dua mekanisme: morfologis dan fisiologis. Perubahan morfologis dihasilkan oleh perubahan ukuran dan densitas kromatofor, sedangkan mekanisme fisologis dikendalikan oleh *melanophore stimulating hormone* (MSH).

Karena sisik terdapat di permukaan tubuh ikan yang selalu mengalami kontak langsung dengan bahan pencemar (logam berat) yang terlarut dalam air, maka sisik ikan dapat digunakan sebagai biomarker pencemaran logam berat. Perubahan lingkungan karena pencemaran logam berat diduga dapat merubah bentuk morfologi sirkulsi dan struktur lain dari sisik ikan, serta ukuran dan densitas kromatorfor. Selain perubahan morfologi, logam berat diduga dapat menganggu kerja *melanophore stimulating hormone* yang berperan mengendalikan perubahan warna pada sisik ikan. Tujuan dari penelitian ini adalah menginvestigasi pengaruh cemaran Cd terhadap perubahan warna dan struktur sisik ikan (meliputi sirkuli, ukuran dan kepadatan kromatofor) serta gangguan MSH yang mengedalikan perubahan warna sisik ikan.

PRAKATA

Puji syukur kehadirat Allah SWT atas limpahan rahmat dan kasih saying NYA, sehingga penulis mampu menyelesaikan laporan akhir tahun penelitian yang berjudul "Perubahan Struktur, Warna dan Fungsi Melanofor pada Sisik Ikan Nila (*Oreochromis niloticus*) setelah Dipapar dengan Kadmium: Sebagai Bioindikator Potensial Pencemaran Kadmium".

Pada kesempatan ini penulis memberikan ucapan terima kasih kepada semua pihak yang telah membantu, yang tidak dapat penulis sebutkan satu persatu.

Penulis menyadari sepenuhnya bahwa walaupun penelitian ini sudah menghasilkan paper yang dikirim ke jurnal internasional (masih *under review* pertama R1), namun penulis tetap menerima masukan dari pihak lain untuk penyempurnaan hasil penelitian selanjutnya. Akhirnya penulis berharap semoga penelitian ini dapat bermanfaat dan bisa memberi sumbangan pemikiran, dalam kaitannya dengan pencemaran, serta berpartisipasi aktif dalam mensukseskan pembangunan bangsa dan negara.

Surabaya, 12 Nopember 2018

Penulis

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BAB 1. PENDAHULUAN

1.1. Latar Belakang

Pencemaran air menyebabkan dampak negatif terhadap ekosistem perairan, merusak keseimbangan ekologi dan mengurangi kemampuan ekosistem untuk membersihkan dirinya sendiri. Logam berat khususnya kadmium (Cd) dapat terakumulasi dan berdampak toksik bagi organisme akuatik jika konsentrasinya melebihi batas ambang (Nursanti et al. 2017). Konsentrasi logam berat yang tinggi dapat menyebabkan kematian organisme akuatik, sedangkan konsentrasi rendah dapat menyebabkan gangguan pada fungsi fisiologi, aktivitas metabolisme dan reproduksi (Ardiansyah et al. 2012).

Logam berat masuk ke dalam tubuh ikan dan organisme air (udang) melalui beberapa cara yaitu melalui makanan maupun melalui air. Melalui air terutama berlangsung di insang dan kulit. Pengaruh Cd terhadap insang organisme air sudah kami lakukan hasilnya menunjukkan bahwa Cd dapat mengganggu aktivitas osmoreguiasi dan kerusakan insang udang *Macrobrachium sintangese* (Putranto et al. 2014). Nursanti et al. (2017) melaporkan bahwa Cd dapat terakumulasi dalam insang serta mempengaruhi peningkatan kadar *metallothionein* dan merubah struktur insang ikan nila (*Oreochromis niloticus*). Sementara penelitian pengaruh Cd pada sisik ikan belum pernah kami dilakukan. Pada kesempatan ini kami ingin mengetahui bagaimana dampak Cd terhadap sisik ikan. Hal ini perlu kami lakukan mengingat sisik merupakan organ terluar dari ikan yang berhubungan langsung dengan polutan logam berat.

Kulit ikan ditutup oleh sisik yang mempunyai peran penting melindungi tubuh dari abrasi air, gesekan dengan batu karang, kehilangan ion, dan invasi oleh pathogen. Selain itu perubahan warna sisik juga merupakan mekanisme adaptasi terhadap perubahan lingkungan (Hawkes 1974).

Ikan nila (*Oreochromis niloticus*) tubuhnya ditutup oleh sisik sikloid (*cycloid*) (Talwar and Jhingran 1992). Sisik sikloid mempunyai sebuah *focus* dan terbagi ke dalam bagian anterior, posterior dan lateral. Bagian *focus* dikelilingi oleh sirkuli (Esmaeli et al. 2007). Pada bagian posterior sisik terdapat sel yang berisi pigment (kromatofor). Ada dua tipe kromatofor: yang pertama merefleksikan cahaya (missal iridofor) dan yang kedua menyerap cahaya (seperti melanofor dan sianofor) (Fujii 2000). Melanofor berisi pigmen warna hitam dan coklat (Sugimoto et al. 2005). Perubahan warna sisik dapat berlangsung melalui dua mekanisme: morfologis dan fisiologis. Perubahan morfologis dihasilkan oleh perubahan ukuran dan densitas kromatofor.

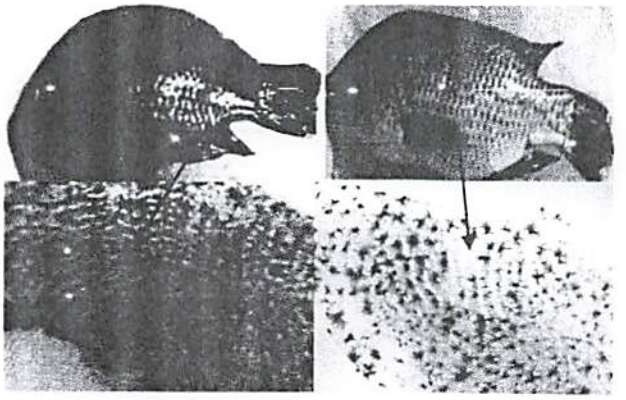
sedangkan mekanisme fisologis dikendalikan oleh *melanophore stimulating hormone* (MSH) (Shiraki et al. 2010).

Karena sisik terdapat di permukaan tubuh ikan yang selalu mengalami kontak langsung dengan bahan pencemar (logam berat) yang terlarut dalam air, maka sisik ikan dapat digunakan sebagai biomarker percemaran logam berat. Perubahan lingkungan karena pencemaran logam berat diduga dapat merubah bentuk morfologi sirkulsi dan struktur lain dari sisik ikan, serta ukuran dan densitas kromatorfor. Selain perubahan morfologi, logam berat diduga dapat menganggu kerja *melanophore stimulating hormone* yang berperan mengendalikan perubahan warna pada sisik ikan. Tujuan dari penelitian ini adalah menginvestigasi pengaruh camaran Cd terhadap perubahan warna dan struktur sisik ikan (meliputi sirkuli, ukuran dan kepadatan kromatofor) serta gangguan MSH yang mengedalikan perubahan warna sisik ikan.

BAB 2. TINJAUAN PUSTAKA

Melanofor merupakan salah satu pigmen warna yang dimiliki kromotofor yang berwarna gelap atau coklat. Epidermis melanofor terletak antara jaringan epidermal dengan dendritik yang tipis yang terbentuk diantara keratonosit schinggga terjadi pengalihan melanosom ke dalam keratonosit (Greaves & Sam 1989). Pada vertebrata pengendapan melanin yang menyebabkan perubahan pigmentasi semua diatur dalam MSH yang bertanggung jawab dalam perubahan pigmen (Greaves &Sam 1989). Vertebrata tingkat rendah pada umumnya memiliki melanofor yang terletak pada dermis, dan dapat berubah karena respons terhadap kondisi biologis dan kondisi lingkungan dan berbeda pada mayoritas vertebrata tingkat tinggi melanofor terletak pada epidermis (Sirisidthi, 2015). Menurut Fuji (2000), warna dasar kromatofor dibagi menjadi 5 kelompok sel yaitu *melanophore* (hitam/coklat), *xanthophore* (kuning), *erythrophere* (merah), *leukophere* atau *guanuphore* (putih).

Perubahan warna yang terjadi pada ikan dipengaruhi oleh letak pengerakan butiran pigmen dalam sel. Pergerakan butiran pigmen kromatofor yang tersebar dalam sel menyebabkan sel tersebut dapat menyerap sinar dengan sempurna sehingga terjadi peningkatan warna sisik menjadi lebih pucat, sedangkan pigmen yang berkumpul didekat nukleus menyebabkan penurunan warna sisik sehingga nampak lebih pucat.



A B

Gambar 2.1. Letak dan Bentuk Melanofor (Van der Salm *et al.*, 2005)
A. Warna sisik gelap (Terdispersi)
B. Warna sisik pucat (Teragregasi)

Perubahan warna pigmen melanofor merupakan salah satu karakteristik dasar hewan, vertebrata terjadi melonosom dalam jaringan derman melonofor (Greaves & Sam. 1989). Perubahan sel pigmen ini disebabkan oleh stress karena lingkungan, kurang sinar matahari, penyakit dan kekurangan pakan (Sulawesty, 1997).

Hormon yang bertanggung jawab terhadap proses pigmentasi ada tiga yaitu *melanosite stimulating hormon* (MSH), *melanin concentrating hormon* (MCH), dan *melatonin* (MT). *Melanosite stimulating hormon* (MSH) diproduksi dibagian tengah lobus dari kelenjar hipofisis, dengan sel target sel kromatofor. *Melanin concentrating hormon* (MCH) diproduksi dibagian ujung lobus dari kelenjar hipofisis dengan sel target pigmen kromatofor. Sedangkan hormon *melatonin* (MT) diproduksi dihipofisis, sel target sel pigmen kromatofor yang menyebabkan granula pigmen berkumpul dalam sel.

Fisiologi perubahan pigmen pada ikan seringkali dibagi dalam dua tipe, yang pertama respon warna dalam kromatofor yang dipengaruhi secara langsung oleh cahaya, tipe lain adalah respon pigmen dalam kromatofor dipengaruhi oleh kontrol dari sistem saraf dan sistem hormon (Fuji, 2000). Perubahan melanofor yang diakibatkan oleh lingkungan terjadi ketika ikan nila berinteraksi dengan logam berat (kadmium) dilingkungan. Kadmium masuk melalui berbagai cara melalui insang, organ pencernaan dan sisik. Pada sisik masuk melalui jaringan dermal sehingga mengalami akumulasi dan menyebabkan sisik mengalami perubahan struktur karena banyak kadmium yang masuk malalui sisik. Ketika struktur berubah dalam sisik protein spesifik dimembran sel menuju ke sitoplasma mengakibatkan meningkatkan ROS dan mengganggu serta merubah aktifitas struktural melanosoma (Organel yang berisi melanin) dalam melanofor (Lenquist, 2010). Meningkatnya ROS *(Reactive Oxygen Species)* dalam pada sisik menyebabkan tidak aktifnya *G-Protein-coupled receptors* (GPCRs) yang merupakan *resptor melakortin-1* (MCIR) yang terletak pada permukaan melanosite (Tsamali M *et al.*,2002). Reseptor melanokortin terdiri atas 317 asam amino yang merupakan resptor untuk homon *melanocyte stimulating hormone* (MSH) (Slominski, 2004). Adanya respon *α-adrenoreseptor* atau *β-adrenoceptor* maka akan terjadi menyebabkan menurunkan aktifitas enzim *adenilsiklase*. Sehingga menyebabkan menurunnya aktifitas cAMP dan peningkatan penyerapan Ca^{2+}. Menurunnya aktivitas cAMP menyebabkan agregasi melanofor, butir pigmen berkumpul pada satu titik (teragregasi) sehingga keseluruhan tampak pucat (Fuji, 2000; Natalia *et al.*, 2009)

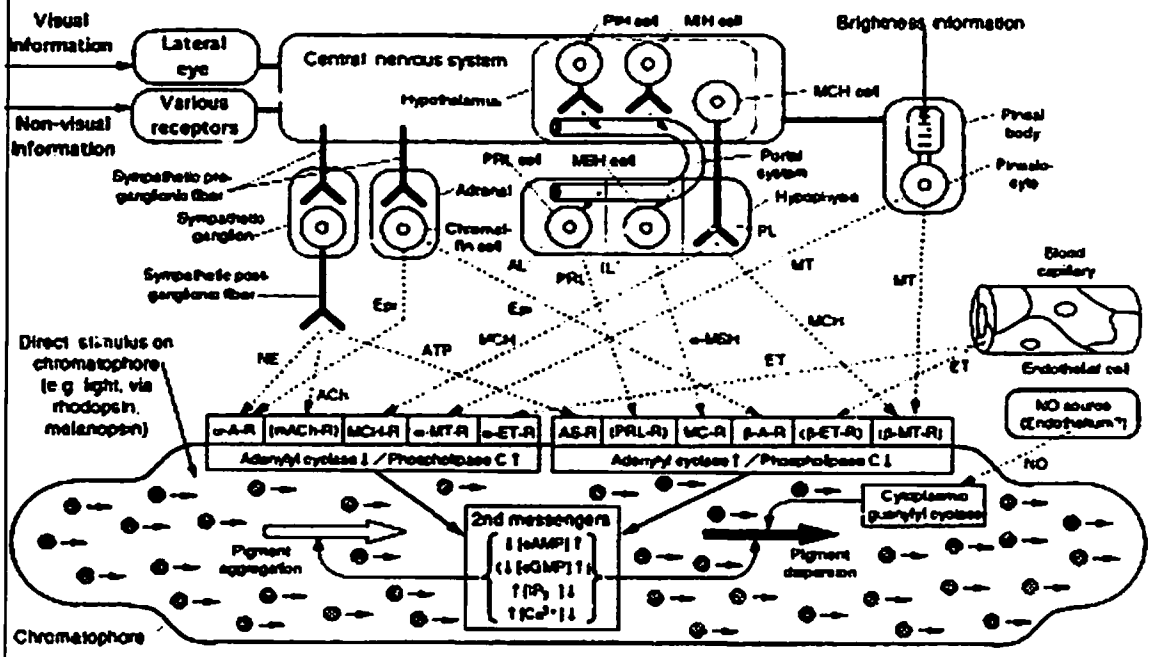
ROS *(Reactive Oxygen Species)* adalah senyawa pengoksidasi turunan oksigen yang bersifat sangat reaktif yang terdiri atas kelompok radikal bebas dan kelompok nonradikal. Kelompok radikal bebas antara lain *superoxide anion* (O2), *hydroxyl radicals* (OH-) dan peroxyl *radicals* (RO_2-). Kelompok yang tidak termasuk radikal bebas *hydrogen peroxide* (H_2O_2) dan *organik peroxides* (ROOH) (Halliewell and Whiteman, 2004). Senyawa oksigen reaktif ini dihasilkan dalam proses metabolisme oksidatif dalam tubuh misalnya pada prosesoksidasi makanan menjadi energi. *Reactive oxygen species* (ROS) secara biologis dan paling banyak berpengaruh pada sistem produksi antara lain *superoxide anion* (O^2), *hydroxyl radicals* (OH^-), *peroxyl radicals* (RO^{2-}) dan *hydrogen peroxide* (H_2O_2) (Tramallen, 2008).

Dampak negatif senyawa tersebut timbul karena aktivitasnya, sehingga dapat merusak komponen sel yang sangat penting untuk mempertahankan integritas sel. ROS yang terbentuk dapat memulai suatu reasksi berantai yang terus berlanjut sampai ROS itu dihilangkan oleh ROS yang lain atau sistem antioksidan (Wijaya, 1996).

Akumulasi proses dimana logam berat masuk dalam organ tertentu akibat dari pencemaran lingkungan. Akumulasi kadmium pada organ dan sisik ikan nila dapat terjadi karena adanya kontak antara medium yang mengandung kadmium dengan ikan (Budiman *et al.*. 2012).Masuknya kadmium ke dalam tubuh ikan melalui tiga cara melalui makanan, insang dan difusi pada permukaan kulit yaitu sisik (budiman *et al.*, 2012). Inti sisik ditutupi dengan sisik sikloid yang merupakan turunan dermal. Sisik merupakan turunan dari dermal yang terlapisi oleh dentin dan pada sisi terluar dari sisik tersebut terlapisi oleh enamel aselular yang merupakan epidermis. Pada daerah basal mengandung melanofor dengan pigmen melanin kehitaman, yang memberikan warna pada ikan.Efek kadmium yang ditimbulkan akibat pencemaran kadmium memiliki pengaruh biologis yang sangat panjang dan waktu yang lama setelah bioakumulasi.

Melanosite stimulating hormon salah satu hormonal yang diketahui bisa mengendalikan perubahan warna pada ikan. Hormon *melanosite stimulating hormon* (MSH) diproduksi oleh hipofisis intermedia dalam kelenjar pituitari. MSH dihasilkan melalui pembelahan protein prekusor yang disebut POMC (*proopimelanocortin)* (Greaves, 1989). Penelitian tentang respon MSH telah banyak dilaporkan diantaranya spesies *Chondricthyes,* dan *osteichthyes.* Pada hewan (Katak) yang kebanyakan aktif pada malam hari atau kurang cahaya, menyebabkan pigmen terdispersi dikarenakan memproduksi banyak *melanosite stimulating hormon* (MSH) yang mengakibatkan tubuh pada katak menjadi gelap. Mekanisme tersebut regulasi melanosite

stimulating hormon pada melanofor ikan berubah ketika kondisi lingkungan (cahaya, logam) masuk dalam perairan yang menyebabkan *G-Protein Couple* yang menghubungkan reseptor dengan enzim *adenil siklase* atau kanal ion terganggu. Pada peristiwa tersebut menyebabkan menurunkan aktivitas enzim *adenil siklase* dan ion Ca^{2+} meningkat sehingga menyebabkan penurunan siklus cAMP sehingga menyebabkan pigmen melanofor dalam melanosom berkumpul pada satu titik (teragregasi) yang nampak pucat (Fujji, 2000).



Gambar 2.2. Regulasi dispersi dan agregasi pada malanosom
Sumber: (Fujji, 2000)

BAB 3. TUJUAN DAN MANFAAT PENELITIAN

Tujuan dari penelitian ini adalah menginvestigasi pengaruh cemaran Cd terhadap perubahan warna dan struktur sisik ikan (meliputi sirkuli, ukuran dan kepadatan kromatofor) serta gangguan MSH yang mengedalikan perubahan warna sisik ikan.

Manfaat dari penelitian ini adalah:

1) Sebagai informasi ilmiah mengenai pengaruh pemaparan kadmium terhadap sisik ikan nila (*Oreochromis niloticus*)
2) Sebagai informasi ilmiah mengenai pengaruh kadmium terhadap MSH ikan nila (*Oreochromis niloticus*) pada salinitas berbeda.
3) Mengetahui pengaruh pemaparan kadmium berpengaruh terhadap kadar kadmium dalam sisik ikan nila (*Oreochromis niloticus*).
4) Mengetahui konsentrasi kadmium berapakah yang dapat menimbulkan perubahan MSH ikan nila (*Oreochromis niloticus*).
5) Mengetahui berapakah kombinasi konsentrasi dan salinitas berbeda yang dapat menimbulkan perubahan MSH dalam ikan nila (*Oreochromis niloticus*).

BAB 4. METODE PENELITIAN

4.1. Tempat dan Waktu Penelitian

Penelitian dilakukan di Universitas Airlangga, Fakultas Sains dan Teknologi, Departemen Biologi, Surabaya, pada periode tahun 2018 dan 2019.

4.2. Hewan Percobaan

Ikan nila (*Oreochromis niloticus*) strain Jawa Timur dengan nama local Jatimbulan yang berukuran 12-14 cm diperoleh dari tempat pemeliharaan ikan komersial di Pasuruan Jawa Timur. Sebelum digunakan dalam percobaaan ikan diaklimasi selama 1 minggu dengan kondisi laboratorium pada salintas berbeda (0, 5, 10 dan 15 ppt). Selama proses aklimasi, air diganti setiap dua hari dan diberi aerasi untuk menjaga agar kadar oksigen terlarut tersedia secara layak bagi ikan nila. Selama aklimasi ikan diberi makan berupa makanan buatan yang berbentuk butiran (pelet) setiap dua hari sekali. Kotoran dan sisa makanan yang tidak dimakan oleh ikan diambil dari tempat pemeliharaan dengan cara disifon setiap hari.

4.3. Penilitian Tahap I

43.3.1. Pembuatan larutan induk timbal

Membuat larutan induk kadmium sebesar 1000 ppm yaitu dengan cara mencampurkan 2.744 g Cd(NO3)2.4H2O (Merck, Darmstadt, Germany) dengan aquademineral sebanyak 1 L, kemudian diaduk merata sampai tidak terdapat endapan.

4.3.2. Tahap pelaksanaan penelitian

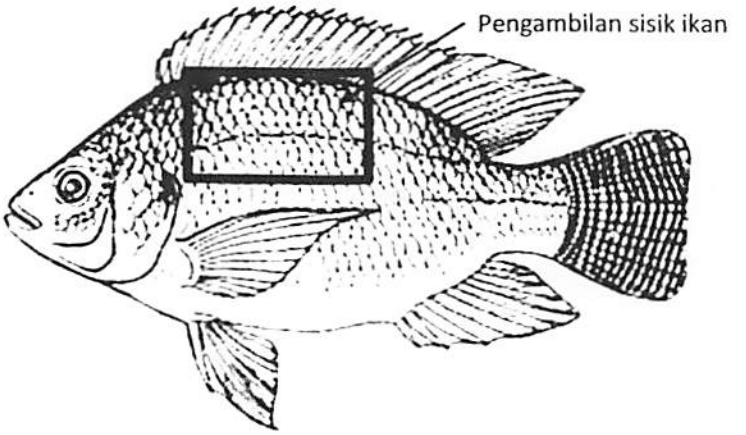
4.3.2.1. Tahap pemaparan logam berat Cd

Pemaparan Cd mengikuti hasil penelitian yang telah dilakukan oleh Nursanti et al. (2017). Sebanyak 10 ekor ikan nila dipapar pada media uji yang mengandung konsentrasi timbal sebesar 0 (control), 2,5 dan 5 ppm masing-masing pada salinitas berbeda (0, 5, 10, 15 ppt) selama 7 hari. Pada masa pemaparan, ikan diberi makanan berupa pelet dua hari sekali dan dilakukan pengamatan terhadap kondisi air pada media uji yaitu pH, suhu, dan oksigen terlarut (DO). Setelah tujuh hari pemaparan ikan nila diambil sampel kulit dan sisiknya untuk dilakukan penelitian lebih lanjut.

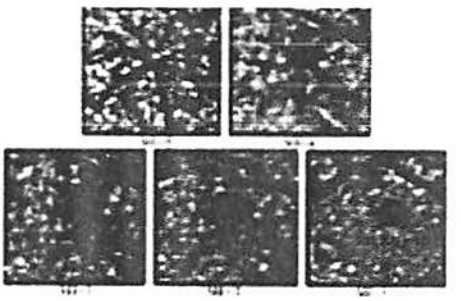
4.4.2. Pengambilan dan analisis morfologi kulit dan sisik ikan nila

Kulit dan sisik diambil dari bagian punggung ikan di bawah sirip dorsal di atas garis linea literalis dengan forcep pada kedua sisinya seperti terlihat dalam Gambar 4.1. Sisik kemudian disikat halus, didehidrasi berturut turut dengan 30, 50, 70 dan 90% ethanol dan dikeringkan dengan kertas tissue (Esmaeili et al. 2012). Sisik yang sudah bersih dan kering kemudian diamati dengan menggunakan mikroskop cahaya untuk mengetahui sebaran dan struktur melanoformya. Indeks melanofor dievaluasi dengan menggunakan metode yang disampaikan oleh Sita (2016), dengan menggunakan panduan seperti disajikan pada Gambar 4.2. Populasi melanofor diamati pada bagian posterior sisik dengan mikroskop cahaya dan dihitung sebagai jumlah kromatofor per mm^2.

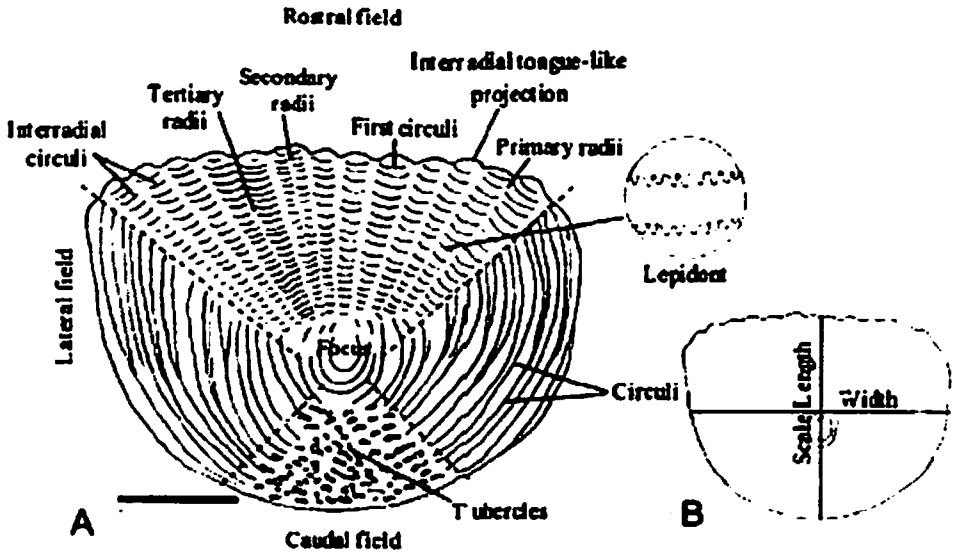
Sebelum mengamati struktur mikro, sisik diletakkan pada sebuah aluminium stub dan dicoating dengan emas. Perubahan struktur mikro seperti yang terdapat Gambar 4.3 diamati dengan seksama dengan menggunakan scanning electron microscope (SEM).



Gambar 4.1. Lokasi pengambilan sisik ikan



Gambar 4.2. Indeks melanofor (5-1) digunakan untuk mengukur respons melanofor dalam studi ini



Gambar 4.3. Stuktur mikro sisik ikan yang akan diamati dengan SEM

4.4.3. Uji kadar Cd dalam sisik ikan

a. Preparasi uji kadar Cd (Asmysari et al. 2013).

1) Mengambil beberapa isik ikan yang masih segar sebanyak 0,5 gram
2) Menambahkan akuades ke dalam blender untuk destruksi secara fisik
3) Destruksi kimiawi dilakukan dengan memasukkan insang ikan ke dalam labu digesti dan ditambahkan 5 mL HNO_3 pekat dan H_2SO_4 pekat
4) Larutan tersebut dipanaskan pada suhu ± 60 °C selama 30 menit, kemudian didinginkan selama 5 menit.
5) Menambahkan 10 mL HNO_3 pekat, lalu dipanaskan sampai 90°C. Larutan dibiarkan selama 5 menit, lalu ditambahkan 1 mL H_2O_2 sampai warna jernih.
6) Memindahkan larutan ke dalam labu ukur 100 mL dan ditambahkan dengan akuades sampai 100 mL. Larutan siap diukur kadar logamnya dengan Spektrofotometer Serapan Atom

b. Pengukuran kadar Cd

Pengukuran kadar logam Pb pada insang dilakukan dengan Atomic absoption spectrophotometer (AAS).

4.4.4. Analisis Data

Data yang diperoleh dalam penelitian ini dianalisis secara statistik dengan menggunakan software SPSS. Normalitas sebaran data diuji dengan menggunakan uji Kolmogorov-Smirnov dan homogenitas data dianalisis menggunakan uji Levene's test. Untuk mengetahui pengaruh Cd, salinitas dan interaksinya dilakukan dengan menggunakan two-way ANOVA dengan taraf ketelitian α = 0,05. Jika perlakuan berbeda nyata, maka dilakukan uji lanjutan dengan menggunakan uji Duncan.

4.5. Penelitian Tahap II

4.5.1. Hewan Percobaan

Hewan uji yang digunakan dalam penelitian ini ikan nila. Perlakuan selama tahap aklimasi sama seperti penelitian tahap I.

4.5.2. Rancangan Penelitian

Percobaan ini bersifat eksperimental dan rancangan penelitian yang digunakan adalah rancangan acak lengkap (RAL) dengan perlakuan konsentrasi Cd (0, 2,5 dan 5 mg/L) pada masing-masing salinitas (0, 5 10 dan 15 ppt). Pengulangan dilakukan sebanyak 3 kali.

4.5.3. Studi fungsi fisiologi melanofor

Untuk mengetahui dampak logam Cd pada salinitas berbeda terhadap fungsi fisiologi melanofor dilakukan beberapa jenis uji antara lain:

A. Pemeriksaan warna dan fungsi melanofor

Warna ikan mempunyai beberapa fungsi yang berbeda, sebagai kamuflase dan komunikasi (Lennquist et al. 2010). Pigment terdapat dalam sel khusus yang disebut kromatofor. Jika pigmen terdispersi di dalam sel ikan akan tampak berwarna gelap, namun jika pigmen mengumpul ikan tampak pucat. Pergerakan pigmen ini dikendalikan oleh oleh hormone dan neurotransmitter.

Melanophore stimulating hormone (MSH) terikat pada melanocortin receptor yang berperan menstimulasi dispersi warna schingga ikan tampak gelap. Melatonin dapat menginduksi pengumpulan pigmen yang terikat pada mel 1c reseptor (Lennquist et al. 2010).

Untuk mengegtahui fungsi fisiologis melanofor dilakukan tahapan berikut. Melanofor disimpan pada dingin dan gelap dalam 4 jam setelah sampling. Eksperimen dilakukan ditempat gelap kecuali saat pengamatan dengan mikroskop cahaya. Sisik dimasukkan kedalam 96-well plate yang berisi 90 µL media kultur sel/well. Tiga sampai empat sisik kemudian difoto dengan mikroskop inverted. Sepuluh microliter MSH (Sigma Aldrich) ditambahkan sampai konsentrasi final 10 nM agar melaonofor terdispersi. Setelah 30 menit, difoto lagi dan larutan cadmium hingga mencapai 100 nM agar melanofor terkumpul. Setalah 30 menit diambol fotonya lagi. Selanjunya perubahan melanofor dihitung dengan menggunakan indeks Hogben-Slome (Lennquist et al. 2010).

B. Pengukuran konsentrasi MSH

Untuk mengetahui kadar MSH dalam sisik dan serum ikan digunakan metode ELISA. Prosedur pengukuran konsentrasi MSH mengikuti panduan yang diterbitkan oleh perusahan yang memproduksi kit. Kita yang akan digunakan adalah Fish MSH ELISA Kit yang diproduksi oleh Bester Catalog No: BLS0043F1 atau menggunakan Kit dari perusahaan lain yang relevan.

4.6. Analisis Data

Analisis data kadar MSH dilakukan dengan analisis statisik parametrik. Uji normalitas sebaran data dilakukan dengan uji Kolmogorof-Smirnov. Jika data berdistribusi normal, maka dilakukan analisis two-way ANOVA untuk mengetahui perbedaan dari perlakuan pada taraf kepercayaan 95 % (α= 0,05). Dan jika perlakuan berbeda nyata, maka dilakukan uji lanjutan dengan menggunakan uji DUNCAN.

BAB 5. HASIL DAN LUARAN YANG DICAPAI

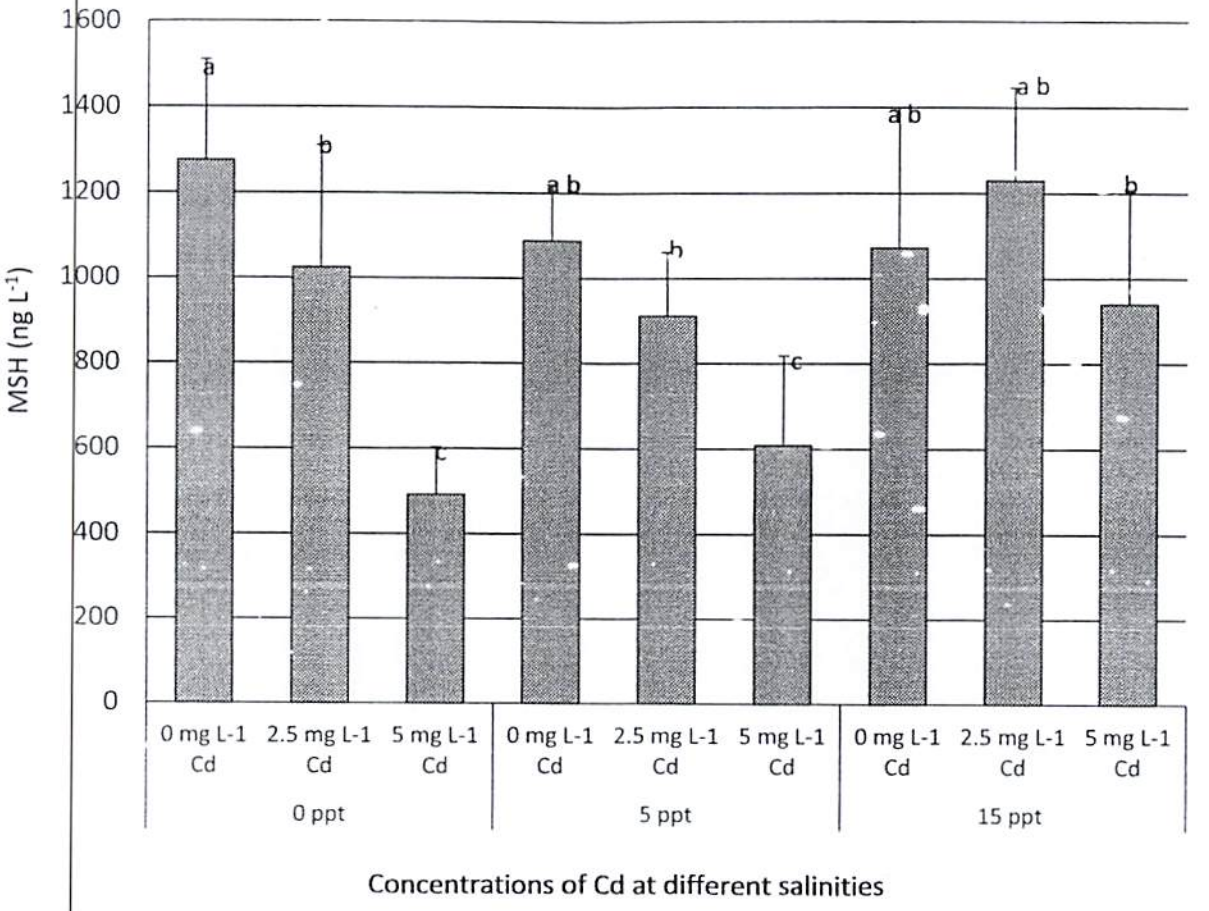


Figure 5.1. MSH of *O. niloticus* exposed to different levels of Cd under different salinities for 7 d. Lowercase letters above bars indicate significant differences (p<0.05, a>b>c). Data are means of five determinations.

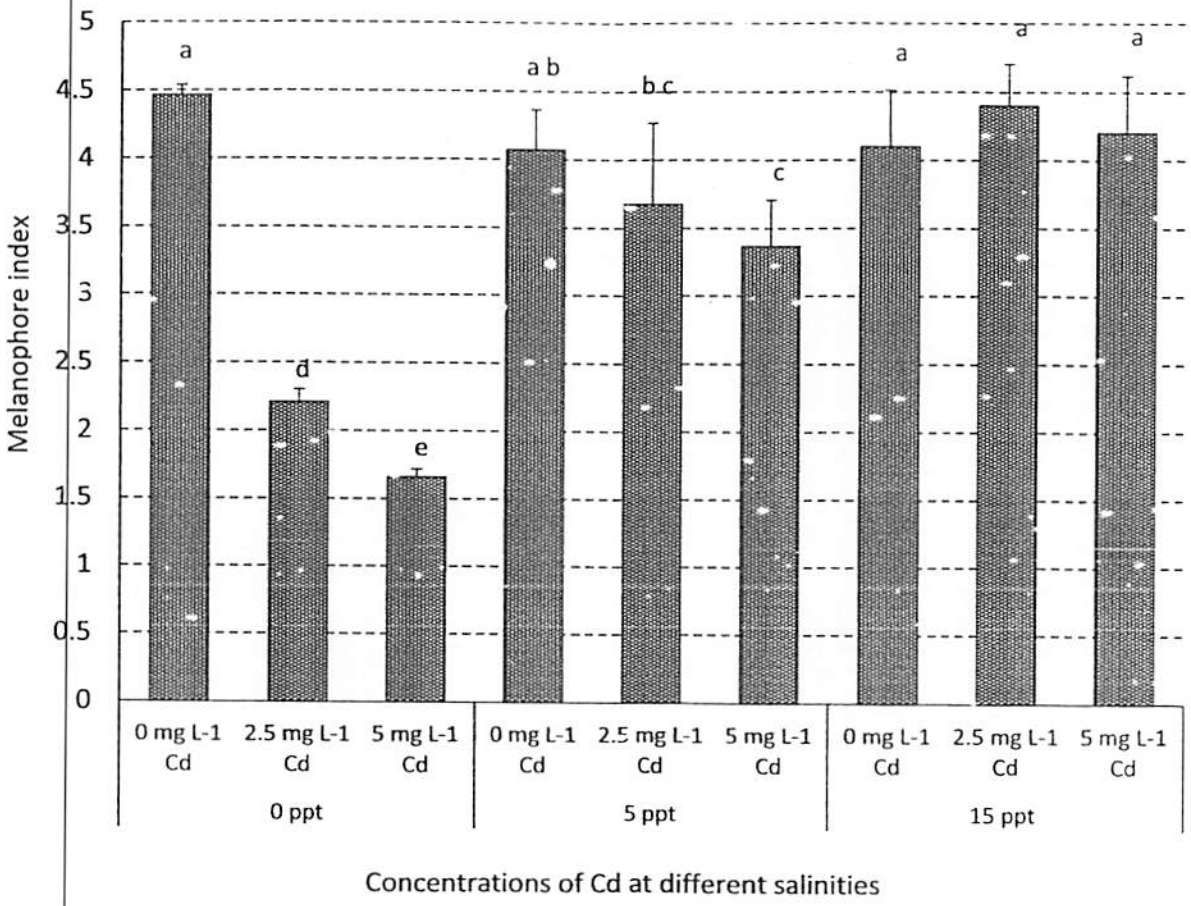


Figure 5.2. Melanophore index of *O. niloticus* exposed to different levels of Cd under different salinities for 7 d. Lowercase letters above bars indicate significant differences ($p<0.05$, a>b>c>d>e). Data are means of five determinations.

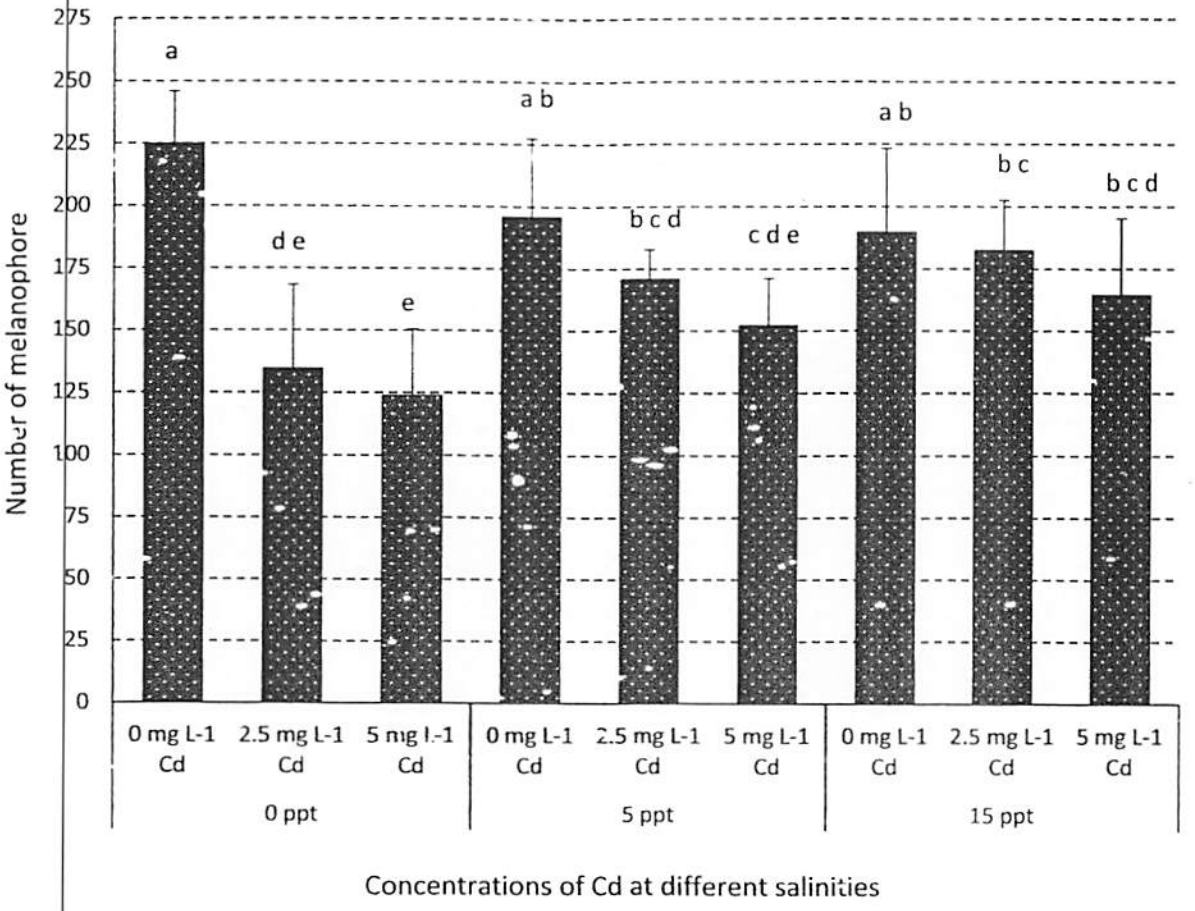


Figure 5.3. Number of melanophore of *O. niloticus* exposed to different levels of Cd under different salinities for 7 d. Lowercase letters above bars indicate significant differences ($p<0.05$, a>b>c>d>e). Data are means of five determinations.

Luaran yang dicapai adalah sebuah paper berjudul:

"Effect of cadmium on plasma melanocyte-stimulating hormone and morphological changes of melanophore in the cichlid fish *Oreochromis niloticus* Linnaeus, 1757 at different salinity levels" yang di*submit* pada jurnal ***Marine and Freshwater Behaviour and Physiology.*** Bukti submit dapat dilihat pada lampiran.

BAB 6. RENCANA TAHAPAN BERIKUTNYA

Rencana tahapan berikutnya adalah:

1. Melakukan penulisan manuscript ke jurnal internasional.
2. Melakukan penelitian lanjutan kadar Cd yang terserap pada pada sisik ikan
3. Melakukan penelitian respons sisik ikan terhadap Cd melalui pendekatan electron mikroskop agar detail perubahan struktur sisik dapat diketahui
4. Melakukan penelitian terhadap respon MSH secara in vivo.

BAB 7. KESIMPULAN DAN SARAN

Kesimpulan:

1. Penelitian ini menunjukkan bahwa efek toksik Cd pada tingkat MSH dan morfologi melanophores menurun dengan meningkatnya salinitas.
2. Chromatophores dapat diusulkan sebagai biomarker paparan Cd dalam ekosistem perairan, karena kepekaan mereka terhadap Cd.

Saran:

1. Melakukan penelitian lanjutan kadar Cd yang terserap pada pada sisik ikan.
2. Melakukan penelitian respons sisik ikan terhadap Cd melalui pendekatan electron mikroskop agar detail perubahan struktur sisik dapat diketahui.
3 Melakukan penelitian terhadap respon MSH secara in vivo

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Effect of cadmium on plasma melanocyte-stimulating hormone and morphological changes of melanophore in the cichlid fish Oreochromis niloticus Linnaeus, 1757 at different salinity levels

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Keywords: Cd, salinity, melanophore, MSH, scale, tilapia

Abstract: Effects of cadmium on melanocyte-stimulating hormone (MSH), melanophore index (MI), menalophore number (MN), as well as microscopic examination of melanocyte in the scale of tilapia (Oreochromis niloticus Linnaeus, 1757) has been evaluated at different salinities. The levels of MSH, MI, and MN were higher in Cd-exposed fish than in control fish (not exposed to Cd) at salinity level of 0 ppt. In media with a salinity level of 5 ppt, fish exposed to 5 mg L-1 Cd presented the lowest levels of MSH, MI and MN. In media with a salinity level of 15 ppt, the levels of MSH, MI and MN in control and Cd-exposed fish were not significantly different. In the media without Cd, the levels of MSH, MI and MN were not significantly different at all salinities. The morphological changes of melanophores were higher in Cd-exposed fish than in control fish at salinity 0 and 5 ppt, respectively. These morphological changes were not significantly different in the control fish at all salinities as well as in fish exposed to 0 to 5 mg L-1 Cd at salinity of 15 ppt. This present study demonstrated that the toxic effect of Cd on the MSH levels and the melanophores morphology decreased with increasing salinity. Further, due to the sensitivity of chromatophores to Cd, therefore, it can be proposed as a biomarker of Cd exposure in aquatic ecosystems.

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Effect of cadmium on plasma melanocyte-stimulating hormone and morphological changes of melanophore in the cichlid fish *Oreochromis niloticus* Linnaeus, 1757 at different salinity levels

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Abstract

Effects of cadmium on melanocyte-stimulating hormone (MSH), melanophore index (MI), melanophore number (MN), as well as microscopic examination of melanocyte in the scale of tilapia (*Oreochromis niloticus* Linnaeus, 1757) has been evaluated at different salinities. The levels of MSH, MI, and MN were higher in Cd-exposed fish than in control fish (not exposed to Cd) at salinity level of 0 ppt. In media with a salinity level of 5 ppt, fish exposed to 5 mg L^{-1} Cd presented the lowest levels of MSH, MI and MN. In media with a salinity level of 15 ppt, the levels of MSH, MI and MN in control and Cd-exposed fish were not significantly different. In the media without Cd, the levels of MSH, MI and MN were not significantly different at all salinities. The morphological changes of melanophores were higher in Cd-exposed fish than in control fish at salinity 0 and 5 ppt, respectively. These morphological changes were not significantly different in the control fish at all salinities as well as in fish exposed to 0 to 5 mg L^{-1} Cd at salinity of 15 ppt. This present study demonstrated that the toxic effect of Cd on the MSH levels and the melanophores morphology decreased with increasing salinity. Further, due to the sensitivity of chromatophores to Cd, therefore, it can be proposed as a biomarker of Cd exposure in aquatic ecosystems.

Keywords: Cd, salinity, melanophore, MSH, scale, tilapia

Introduction

Any change in the aquatic environment must be responded by fish. Scale as the outer structures of the fish's body are in direct and continue contact with pollutants present in the water. The reception of environmental stimuli is mediated by receptors on the surface of the chromatophores under the control of the nervous and endocrine systems (Chaplen et al. 2002; Mousa et al. 2015). There are several types of chromatophores, light reflecting cells, such as iridophores and light absorbing cells, i.e. xantophores, erythrophores, cyanophores and melanophores (Fujii 2000). Melanophores contain melanized organelle termed melanosomes that produce dark colors in the skin of many species (Sugimoto 2002; Sugimoto et al. 2005). There are two major mechanisms involved in color change; physiological color change and morphological color change (Lennquist et al. 2010; Mizusawa et al. 2011). In teleost fish, physiological color change is caused by pigment aggregation or dispersion in skin chromatophores which is controlled by the neuroendocrine and sympathetic nervous systems (Fujii 2000). Melanocyte-stimulating hormone (MSH) binds to the melanocortin receptor which stimulates dispersion of chromatophore pigments in fish scales makes the fish has a dark appearance (Fujii 2000; Lennquist et al. 2010). Melanin-concentrating hormone (MCH) acts on chromatophores and induces pigment aggregation makes the fish looks pale (Baker 1991). Morphological color changes involved both altered rates of pigment synthesis within existing cells as well as the changes in size and density of chromatophores (Healey 1999). These types of color changes are relatively slow, they occur over several days or weeks. In the long-term, these systems also influence survival or apoptosis of the chromatophores and contribute to morphological color change (Sugimoto 1996; Hoglund et al. 2000; Hoglund et al. 2002).

Various tilapia species and strains tolerant to a wide range of salinity levels from 0 to 32 ppt (Sardella et al. 2004; Canonico et al. 2005), however Baroiller et al. (2000) and Soegianto et al. (2017) reported that Nile tilapia *O. niloticus* do not suitable for culture in seawater (37 ppt) because they are tolerable to the salinity between 0 and 15 ppt.

In the natural waters as well as in the aquaculture ponds, fish often encounter both changes in water salinity and heavy metal level (including cadmium). The concentration of Cd in unpolluted environment was < 0.1 µg L^{-1}, however, in heavily polluted waters, Cd concentration can reach 2-16 mg L^{-1} (Cao et al. 2012). This metal enters into the aquatic environment come from both anthropogenic and natural sources. As an abiotic factor, salinity exerts a significant effect on Cd toxicity and accumulation. Toxicity of Cd reduces with increasing salinity of the medium (Erickson et al. 2008; Loro et al. 2012). Salinity affects the metal uptake and its subsequent toxicity by competing with metal ions for binding to biological molecules (Bianchini et al. 2002).

As the scales are most external to the body of the fish, they continuously come in contact with water and the pollutants therein. Therefore these can be very good bioindicators of the state of pollution in water bodies. Chaplen et al. (2002) studied the ability of chromatophores in scale to detect potential environmental toxins such as heavy metals, pesticides and pathogenic bacteria. Allen et al. (2004) examined the use of fish chromatophores as reliable biomarker of aquatic arsenic pollution. Lennquist et al. (2010) studied the effects of long-term exposure of medetomidine (antifouling agent) in the color and melanophore function in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). Kaur and Dua (2015) studied the effects of municipal wastewater in the color changes in scale of freshwater fish *Labio rohita* Hamilton, 1822. The present study was undertaken to investigate the effects of sub-lethal cadmium at different salinity

97 levels of the changes in the pigment granule distribution in scales, as wells as the responses to
98 melanocyte-stimulating hormone (MSH) of tilapia *O. niloticus*.
99
100 ## Materials and methods
101 ***Fish acclimation and experimental design***
102 Tilapia *O. niloticus*, approximately 11.5 ± 0.2 cm length and 15.5 ± 0.7 g weight were purchased
103 from a commercial farm in Pasuruan, East Java Province, Indonesia. The fish were brought to the
104 Department of Biology, Universitas Airlangga, Indonesia, where the experiments were
105 performed. The fish were maintained in a large fiberglass tank (250 L) supplied with a
106 continuous flow of dechlorinate freshwater (FW, 0 ppt) through gravel, sand and sponge filter.
107 Some fish were allowed to acclimatize for 2 weeks in different salinities 0, 5, and 15 ppt. A 5 ppt
108 daily increase of salinity was applied to avoid osmotic shock during acclimation (Soegianto et al.
109 2017). Diluted seawater was made by adding adequate volumes of seawater (SW, 35 ppt) to FW.
110 SW was obtained from the coast adjacent to the university and the FW was obtained from
111 chlorinated municipal tap water. Salinity was measured by using handheld salinity refractometer
112 (Atago, Japan). Throughout the acclimation and experimentation tests fish were fed twice a day
113 with Takari commercial pellets (30% protein, 3% fat and 4% fiber) *at libitum*. The temperature
114 was measured using mercury in glass thermometer (°C), pH using pH meter (Hanna Model HI
115 981502, China), and the dissolved oxygen (DO) using DO meter (Lutron DO 5510, Taiwan).
116 The values of temperature, pH, and dissolved oxygen were 27-29 °C, 7.60 – 8.05 and 7.1 – 7.5
117 mg L^{-1}, respectively. Illumination was provided under a 12 h light, 12 h dark cycle.
118
119

120 ***Effect on MSH, melanophore index and melanophore number***

121 Sub-lethal effect was conducted using the semi-static standard method, with test solutions
122 renewed every 48 h. Fish were exposed for 7 d to nominal Cd concentrations: 0 mg L^{-1} (control),
123 2.5 mg L^{-1} and 5 mg L^{-1}, at salinities of 0, 5, and 15 ppt in 63 L experimental tanks. There were
124 five fish for each test media. The Cd concentrations used in this study was based on the results
125 from Nursanti et al. (2017) (the 96 h LC_{50} of Cd was 7.53 mg L^{-1}), and can potentially be found
126 by fish cultured or lived in the contaminated aquatic environment (Cao et al. 2012). A stock
127 solution of Cd (1000 mg L^{-1}) was prepared by dissolving 2.744 g $Cd(NO_3)_2.4H_2O$ (Merck,
128 Darmstadt, Germany) in 1000 ml of deionized water. Test media were aerated continuously.
129 Uneaten food and debris were removed daily to maintain the water quality of test media.

130 At the end of the exposure period, five fish from each treatment were determined their
131 MSH, melanophore index and melanophore density. We used sandwich-ELISA to measure MSH
132 according to the manufacturer's instructions (Bioassay Technology Laboratory, Shanghai,
133 China). All microtiter wells provided in the ELISA kits were pre-coated with MSH monoclonal
134 antibody. Prior to sampling, fish were anesthetized with 200 mg L^{-1} clove solution (Adhim et al.
135 2017). Blood from each fish was obtained by puncturing the heart using a heparinized syringe.
136 Then, blood samples were introduced to heparinized tubes for the assessment of MSH. The
137 blood samples were then centrifuged for 30 minutes at 3000 rpm at 4°C to obtain the plasma.

138 To measure MSH, 50 µL of standard and 40 µL of sample was added to each well.
139 Immediately 10 µL of anti-Fish MSH antibody was added to each sample well, then 50 µL of
140 streptavidin-horseradish peroxidase was added to each sample well and standard well, mixed
141 well, covered with the plate sealer and incubated for 60 minutes at 37°C. The wells were aspired
142 and washed 5 times with approximately 350 µL wash buffer per well. Any remaining wash

buffer in the well was removed by aspirating or decanting. Further 50 µL of substrate solution A and 50 µL of substrate solution B were added to each well. Then the wells were covered with new sealers and incubated for 10 minutes at 37°C in the dark. To terminate the enzyme reaction. 50 µL of stop solution was added to each well, the blue color will change into yellow immediately. The optical density of solution was determined using an automatic microplate reader (Bio-Rad, model iMark, Japan) at 450 nm. The concentration of MSH was determined using the appropriate standard curves and data were expressed as ng L^{-1}.

The scales were removed from control (unexposed fish) and Cd-exposed fish, with the help of forceps from the second row above the lateral line and below the dorsal fin from both sides. Scales were washed with distilled water, dried and studied in their fresh state under a light microscope (Olympus CX41, Tokyo, Japan) with built-in 12.3 megapixels USB digital camera. Pictures of five scales from 5 fish from each treatment were judged for melanophore index using the method of Hogben (1942). The melanophore index consists of a five-graded scale where 1 means maximum pigment aggregation, and 5 means maximum pigment dispersal. The number of melanophores was quantified in 5 fish from each treatment and in 5 scales from each fish.

Statistical Analysis

All data were expressed as the mean ± standard deviation and verified their normality and homogeneity before used for statistical analysis. The comparisons of the effects of different salinities without Cd and under Cd exposure treatment on MSH, MI, and melanophore number were analyzed using two-way analysis of variance, respectively. When significant differences were detected ($p<0.05$), Duncan's multiple range test was used to determine which treatment is

resulting in significant effect on MSH, MI and melanophore number at a significance level of 0.05.

Results

The concentration of MSH was higher in Cd-exposed fish than in control fish (not exposed to Cd (0 mg L^{-1})) at the salinity level of 0 ppt ($p<0.05$). In media with the salinity level of 5 ppt, the MSH levels of control and fish exposed to 2.5 mg L^{-1} Cd were not significantly different ($p>0.05$), however, their levels were higher than those in fish exposed to 5 mg L^{-1} Cd ($p<0.05$). In media with the salinity level of 15 ppt, the concentrations of MSH in control and Cd-exposed fish were not significantly different ($p<0.05$). In media without Cd, the levels of MSH were not significantly different at all salinities ($p>0.05$) (Figure 1).

The melanophore index (MI) was higher in control than Cd-exposed fish at both salinities of 0 and 5 ppt ($p<0.05$). At the salinity of 15 ppt, the malonophore indexes of control and Cd-exposed fish were not significantly different ($p>0.05$). In media without Cd, the MIs were not significantly different at all salinities ($p>0.05$) (Figure 2).

The number of melanophore in scale was higher in the control than Cd-exposed fish at salinities of 0 ppt ($p<0.05$). At the salinity of 5 ppt, the lowest number of melanophore was noted at the fish exposed to 5 mg L^{-1} Cd ($p<0.05$), while the number of melanophore in the control and fish exposed to 2.5 mg L^{-1} Cd were not significantly different ($p>0.05$). The number of melanophores were not significantly different in the control and Cd-exposed fish at the salinity of 15 ppt ($p>0.05$) (Figure 3).

Microscopic examination showed that the dispersion of the pigment in fish scale melanophores (stages 4 and 5 of MI) was observed in control fish (media without Cd) at all

salinities (Figure 4A, D, and G). Cadmium exposure (2.5 and 5 mg L^{-1}) at the salinity level of 0 ppt caused aggregation of pigment in the melanophore of fish scales (MIs were in stage 1, 2 and 3) (Figure 4B and C). The number of stage 1 MI was higher in fish exposed to 5 mg L^{-1} Cd than 2.5 mg L^{-1} Cd. At salinity level of 5 ppt, we noted that the number of melanophores with reticulated pigment (dispersion) in fish exposed to 5 mg L^{-1} Cd were lower than those in control and 2.5 mg L^{-1} Cd-exposed fish (Figures 4D, E, and F). At salinity level of 15 ppt, no significant changes in the morphology of melanophore were observed in the control and Cd-exposed fish (Figures 4G, H and I). Most melanophores had a reticulated form and we recorded nc punctuated form (aggregation).

Discussion

It is generally accepted that the physiological and morphological color changes in teleost fish proceed simultaneously (Hakkinen et al. 2003). Melanophore indexes (MI) of fish exposed to cadmium (2.5 and 5 mg L^{-1}) were lower than MI of the control fish at the salinity of 0 ppt. Simultaneously the levels of the melanocyte-stimulating hormone (MSH) of Cd-exposed fish were also lower than that of the control fish. Lennquist et al. (2010) reported that the melanin pigment dispersed in response to MSH. They demonstrated that melanophores in isolated scale from fish exposed to medetomidine (antifouling agent) were initially more aggregated than melanophores from the control fish. After MSH addition, there was a significant increase in MI, and subsequent medetomidine addition caused significant aggregation again. The pigment dispersed in response to MSH and aggregated in response to medetomidine. Allen et al. (2004) reported that the depressive effect of MSH during arsenic stress arsenic might be mediated by cAMP. They suggested that microfilaments within the melanocytes act as the transducer between

211 cAMP and pigment granule dispersion. It is well known that in teleost fish, MSH is secreted
212 from the pars intermedia of pituitary and causes pigment dispersion in chromatophores. This
213 system is confirmed to be innervated by the autonomy of sympathetic nerve. Norepinephrine
214 (NE), the neurotransmitters from the sympathetic nerve, induce light-absorbing chromatophores
215 aggregation via binding to the α-adrenoreceptors (Sugimoto 2002). Also, melatonin can induce
216 aggregation via binding to the mel1c receptor (Lennquist et al. 2010). All these receptors are G-
217 protein coupled, and the effects are primarily mediated by changes in intracellular cAMP levels
218 (Aspengren et al. 2008). Decreases in cAMP levels and/or decreases in Ca^{2+} levels within the
219 chromatophores trigger aggregation responses (Sugimoto 2002). We can attribute in the present
220 study that the decrease in MSH during Cd exposure could decrease in cAMP and Ca^{2+} levels
221 within the melanophores and trigger the aggregation of melanin pigment; consequently, decrease
222 the MI value.

223 The number of melanophores differed significantly between the Cd-treatments in media
224 with the salinity of 0 ppt (Figure 3). Fujii (2000) and Sugimoto et al. (2005) demonstrated that as
225 part of the morphological color adaptation, the long-term adaptation of fish to a white
226 background induced a decrease in the number of melanophores. This apoptosis of melanocyte
227 has been suggested to be induced via sympathetic α2-adrenoceptors since paleness and apoptosis
228 were caused by noradrenaline and the α2-adrenoceptor agonist clonidine in isolated skin strips
229 from medaka (Sugimoto et al. 2000; Uchida-Oka and Sugimoto 2001; Lennquist et al. 2010). A
230 decrease in melanophore number in the present study indicated that the concentrations of Cd
231 result in apoptosis of melanocytes. This impact could be severe and irreversible when fish live in
232 the media with the high level of Cd and in the salinity of 0‰ (freshwater).

The impact of Cd on MI, MSH, and number of melanophore decreased with increasing salinity. At the salinity of 15 ppt, we observed that the levels of MI, MSH, and number of melanophore of the control and Cd-exposed fish were not significantly different. At the salinity of 5 ppt, fish exposed to 5 mg L^{-1} Cd presented the lowest levels of MI, MSH, and number of melanophore, however, those levels were not significantly different in the control and in fish exposed to 2.5 mg L^{-1} Cd. All these parameters reached the lowest levels in Cd-exposed fish at the salinity of 0 ppt. It is generally known that the availability of metals to aquatic animals is determined by salinity. An increase in salinity has been shown to cause a decrease in the uptake and toxicity of metals in several aquatic organisms (Blust et al. 1992; Chan et al. 1992; Bervoets et al. 1996). This inverse relationship is usually explained as the result of a decrease in free metal ion concentration, which is the most bioavailable form of metals, with increasing salinity (Mo and Neilson 1993; Rainbow 1995; Putranto et al. 2014). Therefore, we can ascribe that salinity plays the protective role from the toxic effect of Cd to the fish scales. This decreased toxicity might be explained by complexation Cd^{2+} with Cl^- (EL-Hefnawy et al. 2014). Further, salinities did not affect the levels of MI and MSH of the control fish (unexposed to Cd). The balance between secretion of MSH and the dispersion of melanin in the melanophores at all salinities indicating that this species is adapted to the ambient water salinity ranging from 0 to 15 ppt at least for a period of this experiment.

In teleost fish, pigment cells in scales have a number of distinct functions, such as regulation of heat, protection against ultraviolet (UV) radiation, excretion of certain metabolic end product (Hakkinen et al. 2003; Sugimoto et al. 2005), communication with a conspecific (Price et al. 2008; Ligon and McCartney 2016), and camouflage and mimicry to hide from a predator (Adachi et al. 2005; Ryer et al. 2008). However, coloration for protection and

communication seems to be the most important function for skin chromatophores in teleost fish (Sugimoto et al. 2005). If the aquatic environment polluted by Cd and it appears high enough to induce paleness, the fish may face difficulties. In this Cd-polluted aquatic environment, the fish become more conspicuous and vulnerable to hunting predators, susceptible to impaired UV-protection and difficult to communicate with a conspecific. Further, this present study revealed that the chromatophores of fish scale were sensitive to Cd, therefore, it can be proposed as a biomarker of Cd exposure in aquatic ecosystems.

Conclusions

This study showed that the toxic effect of Cd on the MSH levels and the melanophores morphology decreased with increasing salinity. Chromatophores can be proposed as a biomarker of Cd exposure in aquatic ecosystems, because their sensitivity to Cd.

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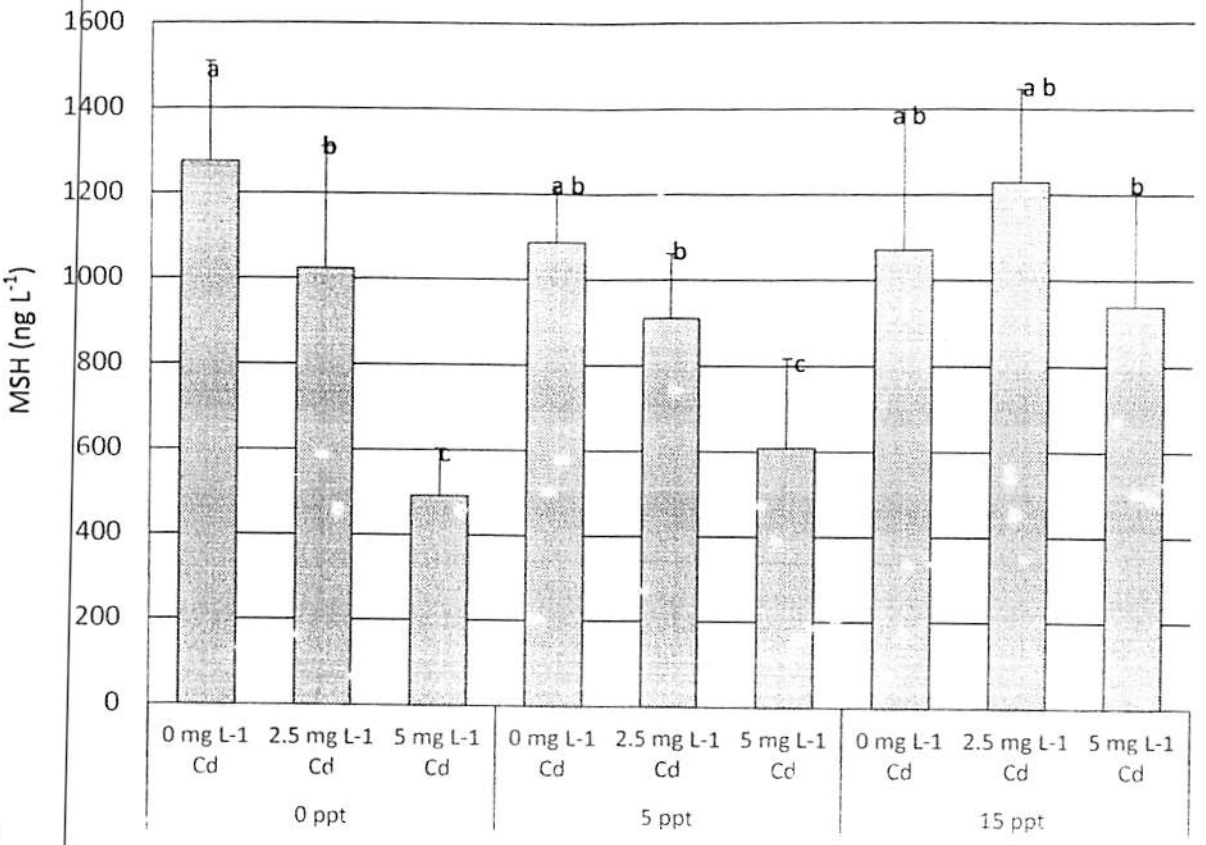

Figure 1. MSH of *O. niloticus* exposed to different levels of Cd under different salinities for 7 d. Lowercase letters above bars indicate significant differences ($p<0.05$, a>b>c). Data are means of five determinations.

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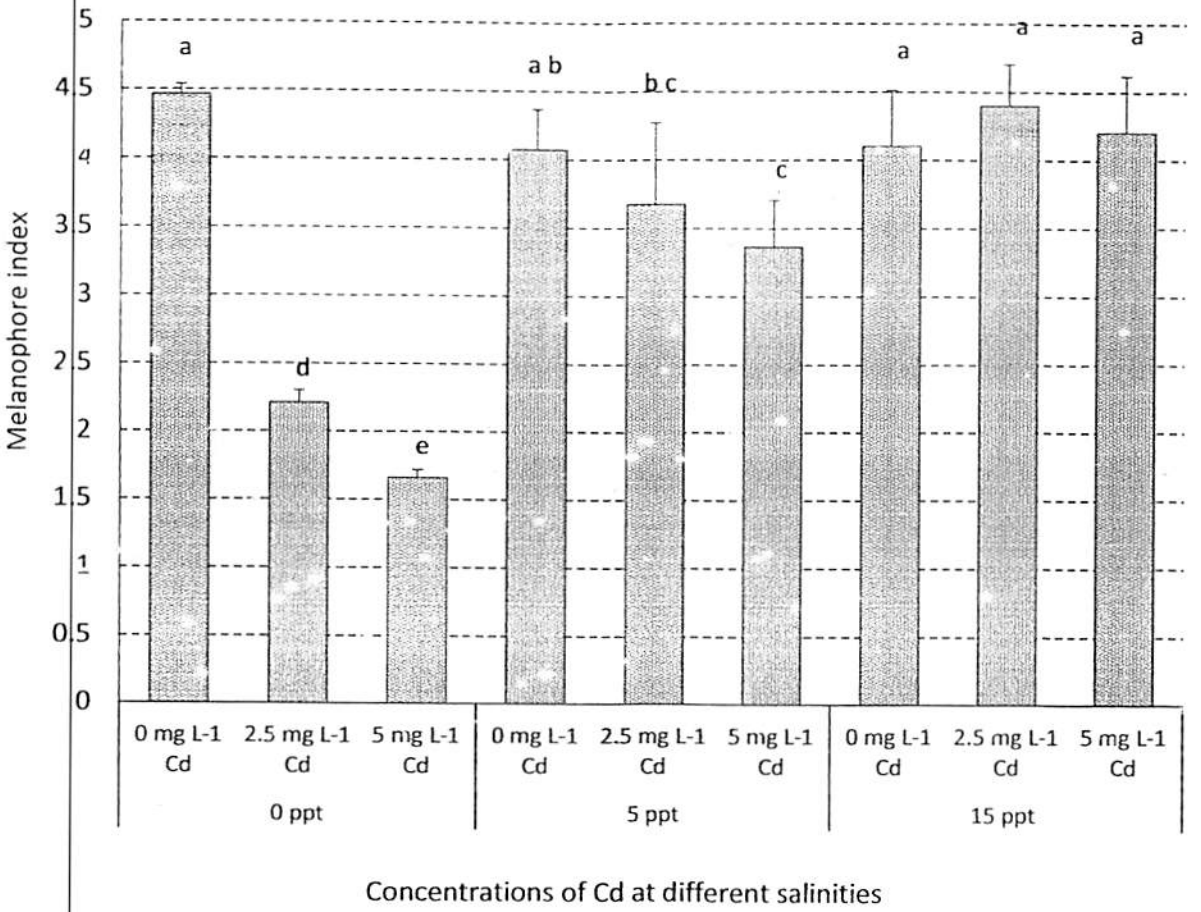

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417

418 **Figure 2.** Melanophore index of *O. niloticus* exposed to different levels of Cd under different

419 salinities for 7 d. Lowercase letters above bars indicate significant differences ($p<0.05$,

420 a>b>c>d>e). Data are means of five determinations.

421

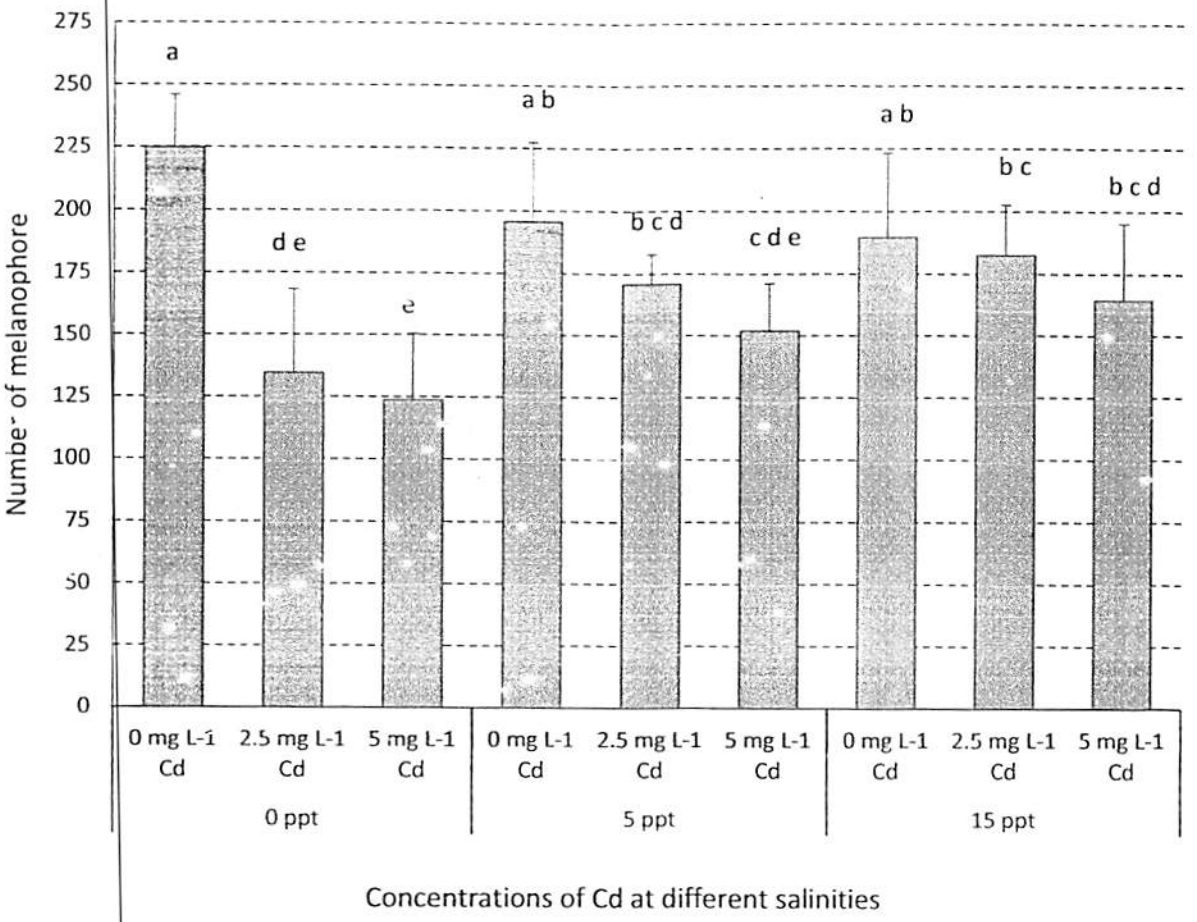

Figure 3. Number of melanophore of *O. niloticus* exposed to different levels of Cd under different salinities for 7 d. Lowercase letters above bars indicate significant differences ($p<0.05$, a>b>c>d>e). Data are means of five determinations.

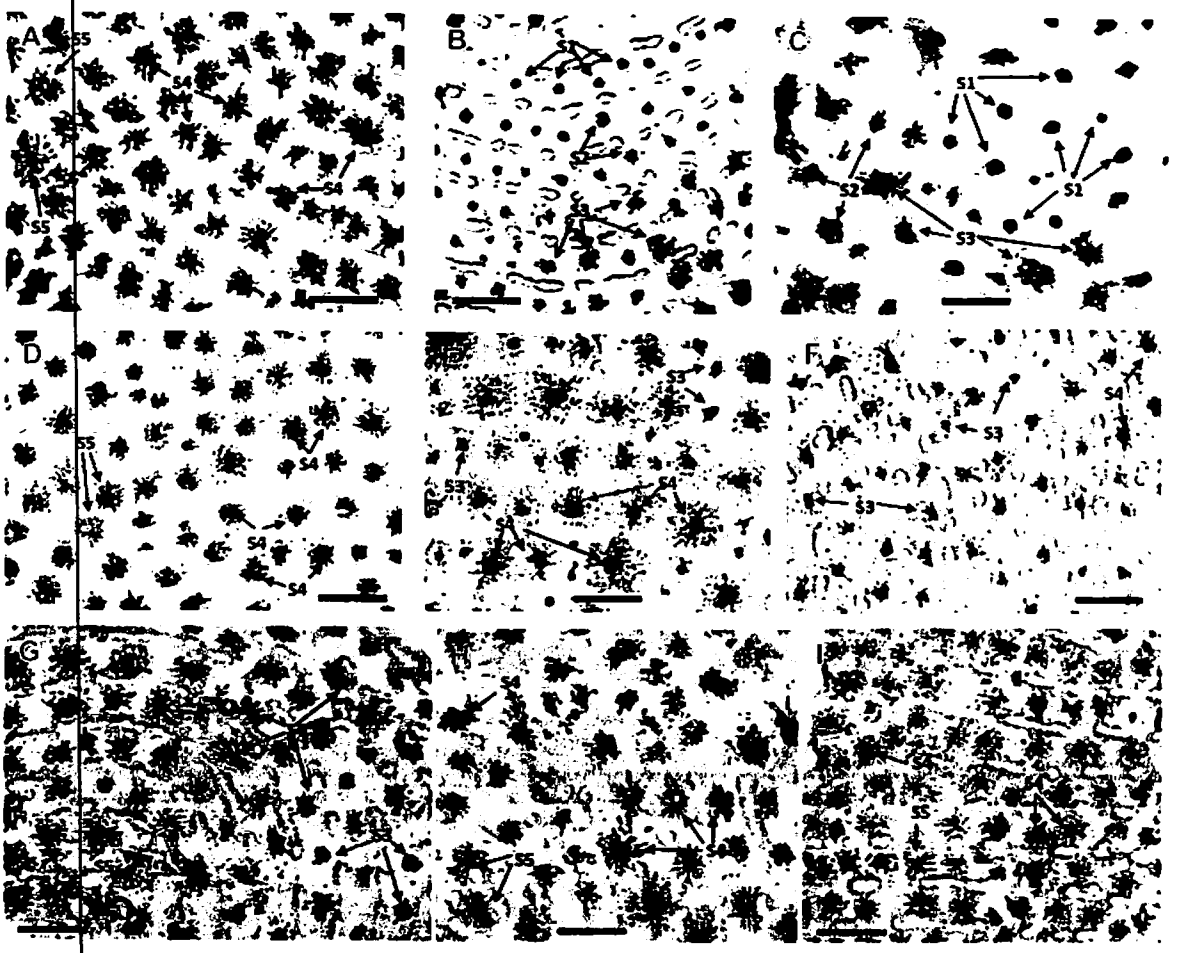

Figure 4. Melanophore of fish scale of ***Oreochromis niloticus*** exposed to different levels of Cd under different salinities for 7 d. Fish exposed to 0 (A), 2.5 (B) and 5 (C) mg L^{-1} Cd at the salinity of 0 ppt. Fish exposed to 0 (D), 2.5 (E) and 5 (F) mg L^{-1} Cd at the salinity of 5 ppt. Fish exposed to 0 (G), 2.5 (H) and 5 (I) mg L^{-1} Cd at the salinity of 15‰. S1-S5 = stage 1 – stage 5 of the melanophore index. Bar size = 0.2 μm.