KEMENTERIAN RISET, TEKNOLOGI, DAN PENDIDIKAN TINGGI
UNIVERSITAS AIRLANGGA
FAKULTAS PERIKANAN DAN KELAUTAN
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# KONTRAK PELAKSANAAN PENELITIAN UNGGULAN FAKULTAS UNIVERSITAS AIRLANGGA TAHUN 2018
## Nomor : 03 /UN3.1.12/LT/2018

Pada hari ini **Selasa** tanggal 10 bulan April tahun **Dua Ribu Delapan Belas**, kami yang bertandatangan di bawah ini :

**1. Prof.Dr.Mirni Lamid,drh.,M.P.** : **Dekan Fakultas Perikanan dan Kelautan** Universitas Airlangga yang berkedudukan di Surabaya, dalam hal ini bertindak untuk dan atas nama Rektor Universitas Airlangga; selanjutnya disebut PIHAK PERTAMA;

**2. Dr. Endang Dewi Masithah,Ir.,M.P.** : Dosen Universitas Airlangga dalam hal ini bertindak untuk dan atas nama **Fakultas Perikanan dan Kelautan** Universitas Airlangga dan selanjutnya disebut PIHAK KEDUA.

PIHAK PERTAMA dan PIHAK KEDUA secara bersama-sama bersepakat mengikatkan diri dalam suatu Kontrak Pelaksanaan Penelitian Unggulan Fakultas Universitas Airlangga Tahun 2018 dengan ketentuan dan syarat-syarat yang diatur dalam pasal-pasal berikut :

**Pasal 1**

Kontrak Pelaksanaan Penelitian Unggulan Fakultas Universitas Airlangga Tahun 2018 ini berdasarkan kepada :

1. Rencana Kegiatan Anggaran Tahunan (RKAT) Fakultas Perikanan dan Kelautan Universitas Airlangga Tahun Anggaran 2018;
2. Surat Keputusan Rektor Universitas Airlangga No. 886/UN3/2018, tanggal 27 Maret 2018, tentang Pelaksanaan Penelitian Internal Universitas Airlangga : Hibah Riset Mandat, Penelitian Unggulan Fakultas dan Penelitian Dosen Pemula Tahun 2018.

**Pasal 2**

(1) PIHAK PERTAMA memberi tugas kepada PIHAK KEDUA, dan PIHAK KEDUA menerima tugas sebagai penanggungjawab pelaksanaan Penelitian Unggulan Fakultas Universitas Airlangga Tahun 2018 dengan judul:

" Kandungan microcystin perairan tambak dalam hubungan dengan keragaman dan dominasi plankton "

(2) PIHAK KEDUA bertanggungjawab penuh dalam pelaksanaan penelitian sebagaimana dimaksud pada ayat (1);

(3) Pelaksanaan penelitian sebagaimana dimaksud pada ayat (1) wajib menghasilkan luaran minimal **satu publikasi** pada Jurnal Ilmiah Internasional terindeks di Scopus minimal Quartil 3 (Q3);

(4) PIHAK KEDUA wajib melaporkan pelaksanaan penelitian dengan melakukan hal-hal berikut:

a. Mencatat semua kegiatan pelaksanaan penelitian pada Buku Harian Penelitian (*logbook*) dan mengisi kegiatan harian secara rutin terhitung sejak penandatanganan kontrak;
b. Menyiapkan bahan pemantauan/monev internal dengan membuat Laporan Kemajuan mengikuti format Panduan Penelitian Unggulan Fakultas dan aturan keuangan yang berlaku;
c. Menyiapkan bahan presentasi monev internal mengikuti format Panduan;
d. Menyiapkan Laporan Akhir penelitian dan mempresentasikannya sebagai pemaparan hasil penelitian;
e. Melaporkan dan menyerahkan bukti luaran penelitian yang dihasilkan serta menyerahkan bukti fisik penggunaan keuangan sebagai pertanggungjawaban keuangan (SPj.)

**Pasal 3**

**PIHAK KEDUA** melaksanakan dan menyelesaikan penelitian sebagaimana dimaksud pada Pasal 2 ayat (1), terhitung mulai tanggal **2 April 2018 s.d. 7 Desember 2018**.

**Pasal 4**

(1) PIHAK PERTAMA memberikan dana untuk kegiatan sebagaimana dimaksud dalam Pasal 2 ayat (1) sebesar **Rp 40.000.000,- (Empat puluh juta rupiah),** dibebankan pada Rencana Kegiatan Anggaran Tahunan (RKAT) Fakultas Perikanan dan Kelautan Universitas Airlangga Tahun Anggaran 2018;

(2) Dana pelaksanaan penelitian ini dibayarkan oleh PIHAK PERTAMA kepada PIHAK KEDUA secara bertahap, dengan ketentuan sebagai berikut:

(a) Pembayaran tahap pertama sebesar 70 % dari total bantuan dana yaitu 70% X Rp.40.000.000,- = Rp 28.000.000,- (Dua puluh delapan juta rupiah) dibayarkan oleh PIHAK PERTAMA kepada PIHAK KEDUA setelah penandatanganan kontrak;

(b) Pembayaran Tahap Kedua sebesar **30 %** dari total bantuan dana kegiatan yaitu 30% X Rp. 40.000.000,- = Rp 12.000.000,- (Dua belas juta rupiah) dibayarkan setelah PIHAK KEDUA menyelesaikan pekerjaan dan menyerahkan semua berkas kepada PIHAK PERTAMA, berupa:

- Laporan Kemajuan Pelaksanaan Penelitian sebanyak 2 (dua) eksemplar paling lambat **15 Agustus 2018**;
- Laporan Akhir Hasil Pelaksanaan Penelitian sebanyak 5 (lima) eksemplar paling lambat **7 Desember 2018**;
- Softcopy Abstrak dan Artikel Ilmiah berdasarkan Laporan Akhir Penelitian Unggulan Fakultas ;
- Softcopy Laporan Akhir Hasil Penelitian Unggulan Fakultas dan Rekapitulasi Keuangan 100% dalam format pdf.
- Laporan/bukti fisik penggunaan keuangan (SPj.) 100% sebanyak satu eksemplar eksemplar paling lambat **7 Desember 2018**;
- Bukti luaran yang dihasilkan berupa paper/Artikel Ilmiah yang telah terpublikasi *(publish/accepted)* di Jurnal Internasional terindeks Scopus Minimal Quartil 3 (Q3) paling lambat **17 Agustus 2019**.

(3) PIHAK KEDUA bertanggungjawab mutlak dalam pembelanjaan dana tersebut pada ayat (1) sesuai dengan proposal kegiatan yang telah disetujui dan berkewajiban untuk menyampaikan semua bukti-bukti pengeluaran dengan jumlah dana yang diberikan oleh PIHAK PERTAMA.

**Pasal 5**

Perubahan terhadap susunan Tim Pelaksana dan Substansi Pelaksanaan Penelitian Unggulan Fakultas dapat dibenarkan apabila telah mendapat persetujuan tertulis dari Dekan Fakultas Perikanan dan Kelautan Universitas Airlangga.

**Pasal 6**

(1) Laporan hasil pelaksanaan penelitian sebagaimana dimaksud dalam Pasal 2 ayat (1) harus memenuhi ketentuan sebagaimana tercantum pada Panduan Pelaksanaan Penelitian Unggulan Fakultas Universitas Airlangga Tahun 2018;

(2) Apabila sampai dengan batas waktu yang telah ditetapkan PIHAK KEDUA belum menyelesaikan tugasnya dan atau terlambat mengirim laporan Kemajuan dan atau terlambat mengirim laporan Akhir, maka PIHAK KEDUA dikenakan sanksi denda sebesar 1 ‰ (satu permil) setiap hari keterlambatan sampai dengan setinggi-tingginya 5% (lima persen) dari nilai kontrak, terhitung dari tanggal jatuh tempo sebagaimana tersebut pada Pasal 2;

(3) Denda sebagaimana dimaksud pada ayat (2) disetorkan ke Rektor Universitas Airlangga melalui PIHAK PERTAMA;

(4) Apabila PIHAK KEDUA tidak dapat memenuhi kewajiban utama menghasilkan luaran minimal **satu publikasi** di Jurnal Internasional terindeks Scopus atau Thompson Reuters, maka akan diberikan sanksi mengembalikan dana yang telah diberikan secara proporsional.

**Pasal 7**

(1) Apabila PIHAK KEDUA tidak dapat melaksanakan Penelitian Unggulan Fakultas ini, maka PIHAK KEDUA wajib menunjuk pengganti ketua pelaksana Penelitian yang merupakan salah satu anggota tim setelah mendapat persetujuan tertulis dari Dekan Fakultas Perikanan dan Kelautan Universitas Airlangga;

(2) Apabila PIHAK KEDUA tidak dapat melaksanakan penelitian ini maka harus mengembalikan dana yang tidak terserap kepada Rektor Universitas Airlangga melalui PIHAK PERTAMA;

(3) Apabila di kemudian hari terbukti bahwa judul Penelitian sebagaimana dimaksud dalam Pasal 2 ayat (1) dijumpai adanya indikasi duplikasi dengan Penelitian lain dan/atau diperoleh indikasi ketidakjujuran/itikad kurang baik yang tidak sesuai dengan kaidah ilmiah, maka kegiatan Penelitian tersebut dinyatakan batal dan PIHAK KEDUA wajib mengembalikan dana Penelitian kepada Rektor Universitas Airlangga melalui PIHAK PERTAMA.

**Pasal 8**

PIHAK KEDUA berkewajiban menyetor pajak ke Kantor Pelayanan Pajak setempat yang berkenaan dengan kewajiban pajak berupa :

1. pembelian barang dan jasa dikenai PPN sebesar 10% dan PPh 22 sebesar 1,5%;
2. pajak–pajak lain sesuai ketentuan yang berlaku.

**Pasal 9**

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan Penelitian Unggulan Fakultas ini diatur dan dikelola sesuai dengan peraturan dan perundang-undangan yang berlaku;

(2) Hasil Hibah Penelitian berupa peralatan dan/atau alat yang dibeli dari kegiatan ini menjadi milik Universitas Airlangga yang dapat dihibahkan kepada institusi/lembaga/masyarakat melalui Surat Keterangan Hibah.

**Pasal 10**

(1) Apabila terjadi perselisihan antara PIHAK PERTAMA dan PIHAK KEDUA dalam pelaksanaan kontrak ini, maka akan dilakukan penyelesaian secara musyawarah untuk mufakat dan apabila tidak tercapai penyelesaian secara musyawarah dan mufakat maka penyelesaian dilakukan melalui proses hukum yang berlaku dengan memilih domisili hukum di Pengadilan Negeri Surabaya;

(2) Hal-hal yang belum diatur dalam perjanjian ini akan diatur kemudian oleh KEDUA BELAH PIHAK.

**Pasal 11**

Surat Perjanjian Pelaksanaan Penelitian Unggulan Fakultas Universitas Airlangga Tahun 2018 ini dibuat rangkap 2 (dua) bermeterai cukup sesuai dengan ketentuan yang berlaku, dan biaya meterai dibebankan kepada PIHAK KEDUA.

| PIHAK KEDUA | PIHAK PERTAMA |
|---|---|
| Dr. Endang Dewi Masithah,Ir.,M.P. | Prof.Dr.Mirni Lamid,drh.,M.P. |
| NIP. 196909121997022001 | NIP. 196201161992032001 |

METERAI TEMPEL A391AAFF044911101 6000 ENAM RIBU RUPIAH

**Pasal 9**

(1) Hak Kekayaan Intelektual yang dihasilkan dari pelaksanaan Penelitian Unggulan Fakultas ini diatur dan dikelola sesuai dengan peraturan dan perundang-undangan yang berlaku;

(2) Hasil Hibah Penelitian berupa peralatan dan/atau alat yang dibeli dari kegiatan ini menjadi milik Universitas Airlangga yang dapat dihibahkan kepada institusi/lembaga/masyarakat melalui Surat Keterangan Hibah.

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Kode/ Nama Rumpun Ilmu : 241 / Bidang Perikanan Lain Yang Belum Tercantum

**LAPORAN AKHIR**
**PENELITIAN UNGGULAN FAKULTAS**

# KANDUNGAN MICROCYSTIN PERAIRAN TAMBAK DALAM HUBUNGAN DENGAN KERAGAMAN DAN DOMINASI PLANKTON


TIM PENGUSUL

Dr. Endang Dewi Masithah, Ir. MP. (NIDN : 0012096902)
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**FAKULTAS PERIKANAN DAN KELAUTAN**
**UNIVERSITAS AIRLANGGA**
**NOPEMBER 2018**

## HALAMAN PENGESAHAN PENELITIAN UNGGULAN FAKULTAS

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| Tahun Pelaksanaan | : Tahun ke-1 dari rencana 1 tahun |
| Biaya Tahun Berjalan | : Rp. 40.000.000,- |
| Biaya Keseluruhan | : Rp. 40.000.000,- (Empat Puluh Juta Rupiah) |

Surabaya, 28 Nopember 2018

Mengetahui,
Dekan Fakultas Perikanan dan Kelautan

Prof. Dr. Mirni Lamid, drh., MP.
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Ketua Peneliti,

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# RINGKASAN

Plankton merupakan organisme renik yang tidak bisa dipisahkan keberadaannya dalam budidaya ikan. Diantara plankton yang ada, Cyanophyceae merupakan golongan plankton dengan jenis-jenis yang merugikan, diantaranya Microcystis, Anabaena, Osscillatoria dan Planktothrix. Disamping menghasilkan geosmin penyebab bau tanah pada ikan, juga menghasilkan racun microcystin penyebab stress oksidatif dan kerusakan hati baik pada organisme budidaya maupun manusia yang mengkonsumsi. Hal ini perlu menjadi perhatian, terutama terkait peningkaan produksi berkelanjutan serta keamanan pangan.

Berdasar pengalaman petambak, munculnya Cyanophyceae di tambak, akan diikuti dengan serangan whitespot diseases yang membawa dampak pada kematian massal udang. Selain faktor alam, berkembangnya Cyanophyceae pada tambak juga dipengaruhi tingkat pengelolaan tambak, seperti pemupukan, pemilihan jenis pakan dan penggunaan probiotik.

Penelitian terdahulu (tahun 2017) mendapatkan bahwa berbagai jenis plankton yang telah diisolasi dari tambak. hasil lain juga menunjukkan bahwa pada perairan tambak di Jawa Timur, baik tambak asin maupun tawar, terdeteksi adanya kandungan racun microcystin. disamping itu, pada udang yang dipelihara, juga mengandung mracun microcystin.

Penelitian ini bertujuan untuk : 1) Mengetahui bagaimana dinamika keragaman dan kepadatan plankton di tambak terutama jenis-jenis plankton penghasil racun microcystin dikaitkan dengan beberapa parameter kualitas air, 2) Mengetahui bagaimana dinamika kandungan racun microcystin di tambak, dikaitkan dengan dinamika keragaman dan dominasi plankton yang terjadi serta 3) Mengetahui berapa nilai parameter beberapa kualitas air, yang menghasilkan keragaman dan kepadatan plankton dengan kadar racun microcystin rendah.

Penelitian ini dilakukan di tambak intensif Banyuwangi, Jawa Timur, dengan parameter identifikasi keragaman, kepadatan dan dominansi plankton; proporsi dan jenis-jenis *Cyanophyceae* pada kondisi tersebut, pengukuran microcystin pada perairan dan produk budidaya ikan/udang serta analisa terhadap berbagai parameter kualitas air (pH, suhu, DO, salinitas, Nitrat, Nitrit, Ammonium, Phospat, Kesadahan, Alkalinitas dan transparansi).

Melalui penelitian ini, diharapkan petambak mendapatkan acuan pengelolaan kualitas air tambak, agar jenis-jenis plankton yang tumbuh adalah jenis-jenis plankton yang tidak berpotensi menghasilkan racun microcystin. Dengan demikian perkembangan teknologi budidaya semakin berkembang untuk menciptakan lingkungan budidaya yang sehat guna memproduksi pangan yang aman dikonsumsi.

Hasil penelitian menunjukkan bahwa dominansi cyanobacteria mempengaruhi konsentrasi toksin microcystin pada sampel yang diamati, yaitu organ insang dan hepatopankreas udang vaname (*Litopenaeus vannamei*) serta air media budidaya udang tersebut. Urutan konsentrasi microcystin paling tinggi terdapat pada hepatopankreas, kemudian disusul dengan air media budaya udang (air tambak) dan insang memiliki konsentrasi microcystin paling rendah. Akumulasi toksin microcystin pada organ insang dan hepatopankreas udang vaname juga menunjukkan adanya perubahan histopatologi pada pengamatan preparat histopatologi organ insang dan hepatopankreas.

## KATA PENGANTAR

Segala puji dan syukur penulis panjatkan kehadirat Allah SWT atas limpahan rahmat dan hidayah-Nya, sehingga penulis dapat menyelesaikan Penelitian tentang KANDUNGAN RACUN MICROCYSTIN PERAIRAN TAMBAK DALAM HUBUNGAN DENGAN KERAGAMAN DAN DOMINASI PLANKTON. Penelitian ini ditujukan untuk mengeksplorasi plankton-plankton berbahaya yang selama ini belum terperhatikan di Indonesia.

Penulis menyadari bahwa penelitian ini belum sempurna, sehingga kritik dan saran yang dapat membangun sangat penulis harapkan demi perbaikan dan kesempurnaan. Penulis berharap semoga karya ilmiah ini bermanfaat dan dapat menjadi informasi bagi banyak pihak.

Surabaya, Nopember 2018

## DAFTAR ISI

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## DAFTAR LAMPIRAN

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# BAB 1. PENDAHULUAN

## 1.1 Latar Belakang

Saat ini Indonesia merupakan negara eksportir udang terbesar mengalahkan India dan Thailand pada tahun 2016 (Kementrian Kelautan dan Perikanan, 2017). Tingginya permintaan konsumen terhadap produk perikanan terutama udang memacu perkembangan industri budidaya udang yang sangat pesat. Selain itu, tingginya nilai produk udang budidaya dan siklus hidup yang relatif singkat menyebabkan sektor ini menarik minat banyak pengusaha (New, 2005). Pada pengembang budidaya udang skala besar dilakukan sistem budidaya intensif. Pada sistem ini dilakukan pengaturan yang ketat terhadap kondisi kolam seperti sistem pengairan, pakan dan perbenihan. Target utama sistem ini ialah jumlah produksi yang tinggi pada area tambak yang kecil, oleh sebab itu dilakukan padat tebar benih yang tinggi dan pemberian pakan dalam jumlah serta kualitas yang tinggi (Fast and Lester, 2012).

Berbagai metode intensifikasi budidaya telah dikembangkan untuk mengelola produktifitas perairan guna meningkatkan daya dukung perairan, mulai mekanisasi seperti penggunaan kincir dan auto feeder, sampai bioteknologi seperti aplikasi bakteri probiotik. Probiotik ditujukan untuk mengarahkan proses degradasi bahan organik agar proses nitrifikasi berjalan dengan baik untuk membentuk rasio N:P perairan yang sesuai untuk pertumbuhan plankton-plankton yang mendukung budidaya ikan/udang.

Pengaturan keragaman dan dominasi plankton menjadi penting karena peranan plankton sebagai produsen primer tidak dapat dihilangkan dari sistem ekologi perairan. Keberadaan dan peranan plankton tidak dapat digantikan oleh jenis mikroorganisme lain. Sebagai produser primer, disamping mensintesis bahan organik dari unsur-unsur hara hasil dekomposisi mikroorganisme menjadi bahan pangan organisme budidaya, peran penting plankton adalah bahwa melalui proses tersebut terjadi penyeimbangan kadar berbagai senyawa di perairan sehingga tidak membahayakan bagi kehidupan organisme budidaya. Berbagai bahan aktif yang meningkatkan imunitas dan kesehatan organisme budidaya juga disuplai dari plankton.

Pada sisi lain, disamping yang menguntungkan, beberapa jenis plankton juga berbahaya bagi kehidupan organisme budidaya karena menghasilkan racun microcystin. Beberapa jenis

plankton dari Cyanobacteria seperti Microcystis, Anabaena, Osscillatoria dan Planktothrix diketahui tidak menguntungkan pada kegiatan budidaya. Disamping menghasilkan geosmin penyebab bau tanah pada ikan, juga menghasilkan racun microcystin penyebab stress oksidatif dan kerusakan hati. Menurut Surono (2015), racun microcystin dapat menyebabkan kerusakan hati dan syaraf bahkan kanker hati bila terakumulai pada organisme. Berdasar informasi petambak, serangan WSSV (White Spot Syndrome Virus) pada udang, terjadi setelah didahului blooming Cyanobacateria (komunikasi pribadi, 2015). Diduga, microcystin yang dikeluarkan oleh Cyanobacteria, turut menyumbang turunnya imunitas udang sehingga terjadi serangan WSSV yang menyebabkan kematian massal.

Kasus kemunculan Cyanobacteria di tambak, walaupun tidak meyebabkan kematian massal, namun menyebabkan terakumulasinya racun hepatotoksin pada udang. Ada beberapa organ tempat bioakumulasi hepatotoksin yaitu hepatopankres, jantung dan otak udang. Penelitian Kankaampaa et.all (2005) menunjukkan adanya peningkatan kadar hepatotoksin berturut-turut sebesar (<5 µg / kg, kemudian 6-20 µg / kg dan 20-80 µg / kg dw ELISA pada sedimen permukaan tambak, daging udang dan hepatopankreas udang. Menurut Zimba, et.all (2006), bioakumulasi microcystin pada tubuh menyebabkan penyakit kanker hati.

Walaupun sudah cukup banyak penelitian tentang Cyanobacteria di Indonesia, namun masih sebatas pada dampaknya terhadap bau tanah pada ikan dan penurunan produksi tambak. Kaitan dengan kadar racun microcystin pada perairan dan dampaknya terhadap organisme air, belum pernah dilakukan di Indonesia. Kasus *blooming* Cyanobacteria di Indonesia yang terekspos adalah di Waduk Saguling, Jawa Barat (Hart, et.al., 2002); Bendungan Sutami, Jawa Timur (Retnaningdyah, dkk., 2010), namun belum mendeteksi kadar racun mycrocistin pada perairan dan organisme.

Penelitian terdahulu (Masithah, dkk., 2017a) mendapatkan data bahwa beberapa jenis Cyanobacteria menghasilkan racun microcystin. Masithah,dkk. (2017b) juga mendapatkan bahwa pada perairan tambak asin dan tawar, mengandung sejumlah microcystin. Demikian juga udang yang dipelihara di dalamnya, dengan kadar pada serta hepatopankreas lebih besar 2 – 3 kali lebih basar dibanding kandungan microcystin pada perairan.

Setelah mendapatkan fakta bahwa berbagai jenis plankton menghasilkan microcystin (Masithah, dkk., 2017a), dan pada tambak di Indonesia juga teridentifikasi terdapat microcystin

(Masithah, dkk., 2017b), maka perlu diteliti dinamika keragaman dan dominasi plankton di tambak intensif dalam hubungannya dengan kandungan microcystin di air tambak dan udang yang dipelihara di dalamnya. Melalui penelitian ini, diharapkan petambak mendapatkan acuan pengelolaan kualitas air tambak, agar jenis-jenis plankton yang tumbuh adalah jenis-jenis plankton yang tidak berpotensi menghasilkan racun microcystin..Dengan demikian perkembangan teknologi budidaya semakin berkembang untuk menciptakan lingkungan budidaya yang sehat guna memproduksi pangan yang aman dikonsumsi.

## 1.2 Perumusan Masalah

Berdasarkan latar belakang diatas, dapat dirumuskan permasalahan sebagai berikut :

1) Bagaimana dinamika keragaman dan kepadatan plankton di tambak terutama jenis-jenis plankton penghasil racun microcystin dikaitkan dengan beberapa parameter kualitas air ?
2) Bagaimana dinamika kandungan racun microcystin di tambak, dikaitkan dengan dinamika keragaman dan dominasi plankton yang terjadi ?
3) Berapa nilai parameter beberapa kualitas air, yang menghasilkan keragaman dan kepadatan plankton dengan kadar racun microcystin rendah ?

# BAB 2. TINJAUAN PUSTAKA

## 2.1 Plankton di Perairan Tambak

Jenis fitoplankton yang sering dijumpai diperairan umum adalah dari kelas Bacillariaphyceae, Chlorophyceae, Chryptophyceae, Chyanophyceae, Dinophyceae, Euglenophyceae, dan Xanthophyceae. Untuk jenis fitoplankton yang sering ditemukan di tambak meliputi alga hijau (Chlorophyta), euglena (Euglenophyta), alga kuning-hijau dan diatom (Chrysophyta), dinoflagellata (Pyrrhophyta), dan alga biru-hijau (Ruttner, 1965).

Ketersediaan nutrisi utama dalam perairan sangat mempengaruhi produktivitas fitoplankton. Pada beberapa lingkungan perairan secara alami telah tersedia komposisi nutrisi yang dibutuhkan fitoplankton untuk pertumbuhan. Produktivitas fitoplankton dapat dipengaruhi oleh ketersediaan satu atau dua nutrisi utama (Boyd dan Tucker 1998). Secara umum, jika disuatu perairan kandungan nutrisinya meningkat, maka akan meningkat pula populasi fitoplankton di perairan tersebut dan sebaliknya (Basmi, 1995).

Keberadaan fitoplankton merupakan komponen yang sangat penting dalam kegiatan budidaya. Hal ini karena fitoplankton adalah makanan alami bagi ikan dan krustasea yang ada di tambak. Selain itu, hasil dari kegiatan fotosintesis fitoplankton dapat memberikan sumber energi utama bagi ekosistem budidaya (Boyd, 1990). Plankton merupakan sumber bbrp mineral yang tidak tergantikan oleh pakan buatan, sumber berbagai enzim dan kofaktor pertumbuhan serta faktor immunostimulan. Namun beberapa jenis dari *Cyanophyta/Cyanobacteria* diketahui dapat memproduksi toksin (racun) (Hoek *et al.*, 1995).

## 2.2 Jenis-jenis Plankton Penghasil Racun Microcystin

Plankton golongan Cyanobacteria yang umum dikenal sebagai microcystin, terkenal sebagi penghasil racun yang berbahaya dalam perairan maupun organisme darat yang memakannya. *Cyanobacteria* memiliki kemampuan untuk berfotosintesis sehingga alga ini dianggap sebagai salah satu pelopor dari kehidupan yang penting di dunia. *Cyanobacteria* mempunyai sifat-sifat yang khas, yang tidak dimiliki oleh tumbuhan lainnya, yaitu tahan kekeringan, tahan panas di dalam air, beberapa jenis lain dapat mengikat molekul $N_2$ dari udara jika di dalam tanah tidak ada nitrat, dapat tumbuh di lingkungan toksik dan dapat tumbuh pada

perairan dengan salinitas tinggi (Thajuddin and Subramanian, 1992). Beberapa anggota dari *Cyanobacteria* telah menunjukkan kemampuannya mengikat nitrogen dari udara dimana kondisi terbaik yang dilakukan oleh *Cyanobacteria* umumnya pada pH 7,0-8,5 (Hardjowigeno, 2007).

*Cyanobacteria* dapat ditemukan pada berbagai kondisi lingkungan, baik lingkungan akuatik maupun terrestrial seperti laut, lumpur, rawa, air tawar, payau, tanah dan bebatuan. Pada umumnya *Cyanobacteria* banyak ditemukan pada perairan tawar dengan pH netral. Meskipun begitu, ada juga *Cyanobacteria* yang hidup pada lingkungan ekstrim seperti sumber air panas, gunung berapi, kutub utara, perairan dengan salinitas yang tinggi dan gurun. Oleh karena itu *Cyanobacteria* dikenal sebagai organisme yang kosmopolit (Graham and Wilcox, 2000). *Cyanobacteria* dapat hidup secara soliter, hidup bebas, sebagai koloni atau filamen. Mereka berukuran mikroskopis tetapi populasinya dapat terlihat, misalnya sebagai bentik, kerak, atau koloni berupa gelatin yang besar (Catherine *et al.*, 2013).

### 2.3 Faktor-faktor yang Mempengaruhi Keragaman dan Dominasi Plankton

Ragam jenis dan dominasi plankton dalam perairan sangat dipengaruhi oleh rasio N:P. Sementara rasio N:P dibentuk oleh berbagai perlakuan dan juga peran kondisi alam. Fitoplankton dalam pertumbuhan dan perkembangbiakan membutuhkan nitrogen dalam bentuk nitrat. Senyawa-senyawa nitrogen dipengaruhi oleh kandungan oksigen terlarut, nitrogen berubah menjadi ammonia saat oksigen terlarut rendah, sebaliknya berubah menjadi nitrat saat oksigen terlarut tinggi (Nybakken, 1992). Sedangkan tinggi rendahnya kandungan fosfat dalam perairan merupakan pendorong terjadinya dominasi fitoplankton tertentu, perairan dengan kandungan fosfat rendah (0,00–0,02 ppm) akan didominasi oleh *Diatom*; pada kadar sedang (0,02– 9 0,05 ppm) didominasi oleh *Chlorophyta* dan pada kadar tinggi (lebih dari 0,10 ppm) didominasi oleh jenis *Cyanobacteria* (Liaw, 1969).

Rasio N:P perairan mempengaruhi dominasi jenis plankton yang tumbuh (Makmur dkk., 2012). Sejalan dengan pendapat Lagus (2009) bahwa keragaman dan komposisi jenis plankton yang dominan di suatu perairan sangat dipengaruhi oleh N:P rasio. Bila N:P rasio diatas 20, perairan akan didominasi oleh diatome; pada N:P rasio berkisar 10, perairan akan didominasi Chlorophyceae (alga hijau seperti *Chlorella*); sedangkan N:P rasio di bawah 10

merupakan lingkungan yang kondusif untuk plankton jenis *Blue Green Algae* (BGA) termasuk *Cyanobacteria*.

Beberapa penelitian menunjukkan suhu optimal untuk pertumbuhan *Cyanobacteria* yaitu 15-35°C, namun beberapa spesies *Cyanobacteria* pernah ditemukan dapat bertahan hidup hingga suhu 72°C di dalam kolam air panas di Taman Nasional Yellowstone (USA). *Cyanobacteria* juga ditemukan pada saat musim dingin dimana suhu udara mencapai suhu 0°C sampai -60°C (Whitton *et al.*, 2002). Hal ini sesuai dengan pendapat Crossetti and Bicudo (2005) bahwa *Cyanobacteria* dapat lebih bertoleransi terhadap kisaran suhu yang lebih tinggi dibandingkan dengan *Cyanobacteria* dan diatom. Rahmadi Aziz (2015) juga menyatakan bahwa *Cyanobacteria* dan *Chlorophyta* merupakan jenis fitoplankton dominan di perairan yang tergenang, namun karena *Cyanobacteria* dapat lebih bertoleransi terhadap kisaran suhu yang lebih tinggi dibandingkan dengan *Chlorophyta* dan *Bacillariophyta* sehingga *Cyanobacteria* lebih mendominasi.

Komunitas fitoplankton pada suatu perairan sangat dipengaruhi factor fisika dan kimia perairan. Hasil penelitian menunjukkan bahwa kandungan hara tambak dataran rendah lebih tinggi dibandingkan tambak dataran tinggi. Salinitas air kolam dataran tinggi lebih tinggi dari pada kolam dataran rendah. Salinitas merupakan faktor utama yang mempengaruhi komunitas fitoplankton. Cyanophyta, seperti *Microcystis* sp., *Merismopedia glauca* dan *Aphanizomenon flosaquae*, mendominasi perairan pada kolam salinitas rendah, sedangkan Diatom seperti *Navicula cryptocephala* dan *Asterionella formosa* di kolam salinitas tinggi (Lie-Min, et all, 2003)

## 2.4 Dampak Negatif Racun Microcystin pada Organisme

*Cyanophyta/Cyanobacteria* menghasilkan sejumlah metabolit melalui proses metabolisme sekunder, beberapa di antaranya bisa sangat berbahaya (toksin) yang dikenal sebagai microcystin. Toksin *Cyanobacteria* (cyanotoxin) dapat dirilis ke dalam air selama mereka mengalami penuaan hingga lisis. Banyak organisme air, terutama ikan, dapat langsung terpapar cyanotoxin yang larut dalam air (Drobac *et al.*, 2016). Toksin ini termasuk hepatotoksin seperti microcystin, nodularin dan cylindrospermopsin, neurotoksin seperti anatoksin, lyngbyatoksin dan β-N-methylamino-$_L$-alanin (BMAA) (Downing *et al.*, 2015).

Cyanotoxin yang dihasilkan oleh spesies plankton air tawar ini terbukti terakumulasi di banyak organisme akuatik (Ibelings and Havens, 2008). Mayoritas studi ini telah berfokus pada microcystins. Selain bioakumulasinya di organisme akuatik, cyanotoxin dapat memiliki efek negatif pada organisme air mulai dari kerusakan hati yang parah, stres oksidatif, menghambat proses pertumbuhan dan keberhasilan reproduksi (Malbrouck and Kestemont, 2006; Ibelings and Havens, 2008).

Dalam lingkungan air, ikan bisa dalam berbagai cara terpapar dengan cyanobacteria dan racun yang dihasilkan, yang dapat mempengaruhi pertumbuhan ikan, perkembangan, histologi, reproduksi serta kelangsungan hidup (Li *et al.*, 2004; Palikova *et al.*, 2004; Deng *et al*,. 2010; Svircev *et al.*, 2015). Paparan cyanotoxin pada ikan dapat terjadi dalam dua cara: cara pertama adalah secara aktif dengan rute oral melalui air yang diminum ikan dan konsumsi sel *Cyanobacteria* dan organisme lain yang telah terakumulasi cyanotoxin. Cara lain yang potensial adalah cara pasif, yairu melalui kontak langsung dengan epitel insang dan air sekitarnya yang mengandung racun. Kedua jenis paparan dapat terjadi pada kondisi alam (Malbrouck and Kestemont, 2006).

Udang yang menelan *Cyanobacteria* beracun telah dilaporkan menginduksi enteritis hemocytic, yaitu penyakit di mana lapisan epitel usus tengah rusak dan lapisan mukosa yang sehat digantikan oleh sel-sel nekrotik dan lapisan hemosit inflamasi (Lightner, 1978). Efek akut dari cyanobacteria dan bioakumulasi toksinnya juga berkontribusi menengah untuk produktivitas jangka panjang budidaya udang melalui penghambatan pertumbuhan hingga kematian, ditambah lagi masalah pada saat pemasaran udang tersebut, terindikasi *off-flavors* (bau lumpur) dan perubahan warna (Kankaanpää *et al.*, 2005).

Secara umum, *Cyanobacteria* menghasilkan sejumlah metabolit melalui proses metabolisme sekunder, beberapa di antaranya bisa sangat berbahaya yang disebut dengan toksin *Cyanobacteria* (cyanotoxin) (Drobac *et al.*, 2016). Toksin ini termasuk hepatotoksin seperti microcystin, nodularin dan cylindrospermopsin serta neurotoksin seperti anatoksin, lyngbyatoksin dan β-N-methylamino-L-alanin (BMAA) (Downing *et al.*, 2015).

Dari sisi keamanan pangan, cemaran microcystin pada manusia bisa ditularkan melalui rantai makanan baik sayuran dari air irigasi tercemar, maupun produk budidaya pada perairan tercemar yang dikonsumsi manusia atau kontak melalui kulit selama kegiatan berwisata.

Menurut Surono (2015), tahun 2003, toksin yang sama ditemukan pada otak 9 orang penderita Alzheimer di Kanada, yang ternyata diketahui bahwa suplemen makanan dari ganggang yang dikonsumi tercemar cyanotoksin penghasil racun. Produk serupa juga ditemukan di Jerman dan Swiss, mengandung mycrocystin-LR. Health Canada, Food Research Division Banting Research Center di Ontario memonitor 100 sampel makanan suplemen Spirulina dalam bentuk pil, kapsul dan bubuk di pasaran, dan menemukan microcyistin pada makanan suplemen dari plankton yang hidup di perairan yang tercemar Cyanobacteria beracun.

Berbagai racun yang diproduksi organisme air tawar dan dan laut dikenal sangat mempengaruhi fisiologi tanaman, ikan, mamalia, dan invertebrata. Kasus kematian massal udang putih di Texas, mendapatkan bahwa pada air, sedimen dan jaringan otot serta hepatopankreas mengandung racun microcystin-LR. Komunitas plankton tersebut didominasi oleh Cyanoprokaryota, terutama *Microcystis aeruginosa* dan *Anabaena* sp. Sampel air dari kolam yang terkena juga mengandung kadar tinggi microcystin-LR (45 µg / l), sedangkan kolam yang berdekatan yang memiliki komunitas plankton berupa diatom dan alga hijau, tidak mengandung racun microcystin (Zimba. Et all, 2006)

# BAB 3. TUJUAN DAN MANFAAT PENELITIAN

## 3.1 Tujuan

Penelitian ini bertujuan untuk :

1) Mengetahui bagaimana dinamika keragaman dan kepadatan plankton di tambak terutama jenis-jenis plankton penghasil racun microcystin dikaitkan dengan beberapa parameter kualitas air ?
2) Mengetahui bagaimana dinamika kandungan racun microcystin di tambak, dikaitkan dengan dinamika keragaman dan dominasi plankton yang terjadi ?
3) Mengetahui berapa nilai parameter beberapa kualitas air, yang menghasilkan keragaman dan kepadatan plankton dengan kadar racun microcystin rendah ?

## 3.2 Manfaat

Penelitian kaitan keragaman dan kepadatan plankton dengan kandungan racun microcystin, belum pernah dilakukan di Indonesia. Berdasar pengalaman petambak, munculnya Cyanophyceae yang diketahui menghasilkan racun microcystin, akan diikuti serangan whitespot diseases yang menyebabkan kematian udang secara massal. Berbagai penelitian untuk memecahkan permasalahan lapang ini dapat dikembangkan dengan mengetahui kaitan keragaman dan kepadatan plankton dengan kadar racun microcystin di perairan pada berbagai tingkat pengelolaan tambak. Dengan demikian, dapat dilakukan manajemen pengelolaan tambak untuk menghasilkan lingkungan budidaya yang sehat guna menjamin keamanan pangan.

# BAB 4. METODE PENELITIAN

## 4.1 Waktu dan Tempat

Penelitian ini dilaksanakan pada bulan April-Nopember 2018. Penelitian dilakukan di tambak intensif Banyuwangi. Analisa plankton dan kualitas air dilakukan di Laboratorium Shrimp Club Indonesia, Banyuwangi. Analisa microcystin di Laboratorium Sentral Ilmu Hayati (LSIH) pada unit Laboratorium Nano, Molecular and Cellular Universitas Brawijaya-Malang, Pembuatan preparat histopatologis di Laboratorium Anatomi Patologi Fakultas Kedokteran, Universitas Brawijaya, Malang.

## 4.2 Materi Penelitian

### 4.2.1 Peralatan Penelitian

Alat-alat yang digunakan dalam penelitian ini adalah DO meter, pH *paper*, Test Kit untuk ammonium, nitrit, nitrat dan fosfat, plankton net, *container* (ember), *haemocytometer*, pipet tetes, mikroskop, obyek glass, cover glass, botol sampel, buret, statif, klem, gelas ukur 100 ml, dan alat bedah (gunting bedah, pisau bedah, dan pinset). Peralatan untuk ELISA yang meliputi *polyestyrene* 96 *well microtiter plate* (*microplate*), micropipet (100-200 µL), *microtube* (1-1,5 mL), *baker glass*, *vortexer* (Bio-Rad, BR 2000), *alat inkubasi shaker* suhu 37°C (HLC Biotech, MHR 13, Germany) dan ELISA *Reader* (Biotrak II *Reader,* 80-2115-82, Biochrom Ltd, Cambridge,UK), serta peralatan histopatologi yang meliputi mikroskop olympus DP71, objek *glass*, cover *glass*, tabung 50 cc, *casette with lid* (MEC-500P), *tissue tek Tec Plus Cryo Console* (Sakura Seiki Co. Ltd., 75-5 Imojiya, Chikuma-shi, Nagano-ken, Japan), inkubasi, *tissue tek* DRS 2000 *Multiple Slide Stainer* (Sakura Seiki Co. Ltd., 75-5 Imojiya, Chikuma-shi, Nagano-ken, Japan), *water bath* 1450 (Sakura Seiki Co. Ltd., 75-5 imojiya, Chikuma-shi, Nagano-ken, Japan), *Semi-automated Rotary Microtome (Leica Biosystem* RM 2245, Wetzlar (Germany).

### 4.2.2 Bahan Penelitian

#### 4.2.2.1 Hewan Uji

Hewan uji yang digunakan adalah udang vaname (*Litopenaeus vannamei*) dengan DOC 94 sebanyak 8 ekor yang diperoleh dari tambak udang vaname. Hewan uji akan diambil organ insang dan hepatopankreasnya, kemudian di preparasi mengikuti protokol yang tertera pada Microcystin ELISA test kit untuk pengukuran konsentrasi microcystin menggunakan uji ELISA serta pembuatan preparat histopatologi.

#### 4.2.2.2 Sampel Air

Sampel air tambak udang vaname digunakan untuk pengamatan cyanobacteria dan kualitas air setiap harinya, dan diakhir penelitian akan dilakukan uji ELISA untuk mengetahui konsentrasi microcystin pada sampel air tambak tersebut.

#### 4.2.2.3 *Enzyme-Linked Immunosorbent Assay* (ELISA)

Bahan yang digunakan untuk pengukuran konsentrasi microcystin menggunakan Microcystin ELISA kit dengan cat no. orb59527 (*Biorbyt, Explore Bioreagents, United Kingdom*) yaitu sampel insang dan hepatopankreas udang vaname, larutan *Phosphate Buffered Saline* (PBS pH 7.4), *ammonium hydrogen carbonate*, *negative control*, HRP *conjugate*, MCY-LR *standard, stop solution, substrate solution*, *wash buffer* dan akuades.

#### 4.2.2.4 Uji Histopatologi

Bahan yang digunakan untuk pembuatan preparat histopatologi adalah sampel insang dan hepatopankreas udang vaname, *Buffer Netral Formalin* (BNF) 10%, parafin cair, alkohol asam 1%, alkohol bertingkat (70%, 80%, 96%, dan alkohol absolut), xylol, *hematoxylin* 560, eosin *trichrome* 515, dan amonia lithium karbonat.

### 4.3 Metode Penelitian

Metodologi penelitian ini menggunakan metode survey atau metode deskriptif yaitu suatu metode penelitian yang ditujukan untuk menggambarkan fenomena-fenomena yang ada, yang berlangsung pada saat ini atau saat yang lampau. Penelitian ini tidak mengadakan manipulasi atau pengubahan pada variabel-variabel bebas, tetapi menggambarkan suatu kondisi apa adanya (Sukmadinata, 2012). Variabel yang diteliti bisa tunggal (satu variabel) bisa juga lebih dari satu variabel (Setyosari, 2010).

### 4.4. Parameter Penelitian

#### 4.4.1 Parameter Utama

Pada penelitian ini parameter utama yang diamati adalah keragaman dan kepadatan cyanobacteria, konsentrasi microcystin pada organ insang, hepatopankreas udang vaname dan air tambak media budidaya udang tersebut. Serta gambaran histopatologis dari organ insang dan hepatopankreas udang vaname.

#### 4.4.2 Parameter Pendukung

Paramater pendukung pada penelitian ini keragaman dan kepadatan fitoplankton serta data kualitas air tambak media budidaya udang vaname selama penelitian berlangsung.

### 4.5 Prosedur Kerja dan Pengumpulan Data

#### 4.5.1 Persiapan Penelitian

Persiapan penelitian dilakukan dengan persiapan alat dan bahan yang dibutuhkan, kemudian melakukan sterilisasi dan standardisasi pada alat-alat yang akan digunakan seperti DO meter, pH *paper*, Test Kit ammonium, nitrit, nitrat dan fosfat, plankton net, *haemocytometer*, pipet tetes, mikroskop, obyek glass, cover glass, botol sampel, gelas ukur 100 ml, *microtube* (1-1,5 mL), *baker glass*, dan alat bedah (gunting bedah, pisau bedah, dan pinset). Alat-alat yang perlu di sterilisasi terlebih dahulu dicuci dengan deterjen, kemudian dibilas dengan air sampai bersih lalu dikeringkan. Setelah kering masing-masing dibungkus dengan aluminium foil. Sterilisasi menggunakan *autoclave* pada suhu 121°C selama 15 menit. Setelah proses sterilisasi, alat dikeluarkan dari *autoclave* (Hardianie, 2013).

#### 4.5.2 Pelaksanaan Penelitian

**A. Pengukuran Parameter Kualitas Air**

Pengukuran parameter kualitas air tambak dilakukan setiap hari yaitu pada pukul 05.00 WIB dan 13.00 WIB. Hal ini dikarenakan pada waktu tersebut merupakan waktu terendah dan tertinggi untuk suhu, pH dan oksigen terlarut pada perairan serta ketiga parameter tersebut disetiap harinya mengalami fluktuasi karena beberapa faktor seperti cuaca (Furtado *et al.*, 2011). Cara yang dilakukan untuk pengambilan sampel air adalah dengan mengikatkan botol sampel pada *seichi disk* dan di masukkan secara horizontal pada tepian tambak hingga kedalaman sekitar 15 cm dari dasar tambak.

Pengaruh suhu karena intensitas cahaya matahari secara langsung terhadap plankton dapat meningkatkan reaksi kimia laju fotosintesis sehingga mengakibatkan ketersediaan oksigen terlarut meningkat dan pH juga mengalami peningkatan karena ada ion hidroksil yang dihasilkan saat proses fotosintesis (Simanjuntak, 2009).

Pengukuran alkalinitas, salinitas, nitrit, nitrat, fosfat, dan amonium juga dilakukan setiap hari. Alkalinitas sangat erat kaitannya dengan pH perairan karena alkalinitas berperan sebagai penyangga, sehingga jika alkalinitas meningkat maka pH akan meningkat pula. Pengukuran alkalinitas dilakukan pada saat pH rendah dan tinggi karena komposisi dari penyusun alkalinitas jumlahnya berbeda (Wilson, 2010). Pengukuran nitrit, nitrat, amonium didasarkan pada kinerja bakteri nitrifikasi dan bakteri denitrifkasi anaerob dalam mengoksidasi perubahan tahap dari $NH_4$-$NO_2$-$NO_3$-$N_2$ dalam perairan (Furtado *et al.*, 2011).

**B. Identifikasi Fitoplankton**

Pengamatan fitoplankton meliputi identifikasi keragaman dan kepadatan dalam tambak. Pengambilan sampel untuk pengamatan fitoplankton dilakukan dengan mengambil sejumlah air tambak menggunakan *container* (ember). Pengambilan sampel plankton pada perairan dangkal dapat dilakukan dengan menggunakan *container* (ember) (Asriyana dan Yuliana, 2012), kemudian disaring dengan plankton net (Dewiyanti *et al.*, 2014).

Sampel air yang sudah diperoleh diberi label yang berisikan tanggal dan posisi pengambilan sampel dan segera dilakukan pengamatan fitoplankton dengan menggunakan mikroskop binokuler perbesaran 100-400x (Nontji, 2008). Pengamatan fitoplankton dilakukan setiap hari pada saat oksigen terlarut rendah dan tinggi untuk mengetahui dinamika kelimpahan plankton karena kelimpahan plankton berfluktuasi secara periodik, dan kelimpahannya tergantung pada unsur hara yang tersedia (Juhar, 2008). Pengambilan sampel dilakukan pada pukul 05.00 WIB dan 13.00 WIB. Penetapan waktu tersebut berdasarkan pada survey yang sebelumnya telah dilakukan di tambak.

Metode yang digunakan dalam identifikasi dan pengamatan cyanobacteria adalah dengan metode perhitungan langsung (*direct counting*) menggunakan *haemocytometer*. Penghitungan kepadatan dilakukan dengan menggunakan *haemocytometer* dan *handtally counter* untuk memudahkan perhitungan. Penghitungan dilakukan menggunakan *haemocytometer* dengan cara mengambil 1 ml air sampel dari 100 ml, kemudian ditutup dengan

gelas penutup dan diamati dengan mikroskop. Rumus penghitungan plankton yang digunakan adalah metode *Small Block* (Satyantini dkk., 2012).

$$N = \frac{nA + nB + nC + nD + nE}{5 \times 4 \times 10^{-6}}$$

Keterangan :

nA, nB, nC, nD dan nE : Jumlah sel fitoplankton pada blok A,B,C,D dan E

konstanta 5 : Jumlah blok yang dihitung

$4 \times 10^{-6}$ : Luas *small block* (A, B, C, D dan E)

**C. Sampling Udang Vaname dan Air Tambak**

Pengambilan sampel udang vaname untuk analisa microcystin dan pembuatan preparat histopatologi menggunakan metode *purposive sampling*. Nasution (2003) menyatakan bahwa metode *purposive sampling* adalah penentuan titik sampling yang dilakukan atas dasar kriteria yang telah ditentukan oleh peneliti dan dianggap bahwa unsur-unsur yang dikehendaki telah ada dalam anggota sampel yang akan diambil, sehingga memungkinkan peneliti menentukan titik-titik sampling sesuai dengan kondisi yang ada pada saat itu.

Sampel udang vaname yang diperoleh kemudian diaklimatisasi terlebih dahulu sebelum dilakukan proses transportasi dalam kondisi hidup ke laboratorium untuk uji ELISA dan pembuatan preparat histopatologis.

Sedangkan sampel air diperoleh dengan cara mengambil sejumlah air tambak menggunakan botol. Sampel tersebut kemudian dibawa ke Laboratorium untuk uji ELISA. Kualitas air sampel dapat dijaga dengan menggunakan suhu 4°C. Kualitas air sampel dapat menurun tergantung penempatan dan lama penyimpanan air sampel (Nontji, 2008).

**D. Pengambilan Sampel Insang dan Hepatopankreas**

Sampel udang vaname (*Litopenaeus vannamei*) tiba di Laboratorium Sentral Ilmu Hayati (LSIH), Universitas Brawijaya – Malang dalam keadaan hidup, kemudian di masukkan dalam wadah sterofoam yang berisikan es batu selama kurang dari 5 menit atau sampai udang tidak terlalu aktif lagi untuk mempermudah proses pembedahan.

Udang vaname dibedah secara hati-hati untuk diambil organ insang dan hepatopankreasnya. Insang dan hepatopankreas yang telah diperoleh langsung dicuci menggunakan larutan *Phosphate Buffered Saline* (PBS pH 7,4). Insang dan hepatopankreas

yang telah dicuci ditimbang masing-masing sebanyak 0,1 gram kemudian dimasukkan pada *tube* 1,5ml dan ditambahkan 1ml larutan *extraction buffer* (0,1M *Ammonium hydrogen carbonate*) menggunakan *blue tip*, mikropipet bervolume 1000 µl.

Organ dan larutan *extraction buffer* dalam *tube* dihomogenkan dengan cara digunting-gunting sampai hancur, kemudian di vortex selama 20 detik dan di *sentrifuge* dengan kecepatan 3000rpm selama 3 menit pada suhu ruang (25°C). Supernatan yang diperoleh dari proses sentrifugasi diambil sebanyak 100 µl secara hati-hati menggunakan *yellow tip* dan dipindahkan ke dalam *tube* 1,5 ml kosong, kemudian di tambahkan 900 µl akuades steril (pengenceran 1:10), hasil pengenceran ini yang akan digunakan untuk ELISA. *Tube* tersebut diberi label dan disimpan dalam *freezer* (-20°C) hingga dilakukan uji ELISA.

**E. Kosentrasi Microcystin**

Konsentrasi microcystin di analisa menggunakan Microcystin ELISA kit yang dilakukan di Laboratorium Sentral Ilmu Hayati (LSIH) pada unit Laboratorium Nano, Molecular and Cellular Universitas Brawijaya - Malang dengan sampel hasil pengenceran supernatan dan akuades (1:10) yang telah disimpan dalam *freezer* sebelumnya. Penyimpanan Microcystin ELISA kit yaitu pada suhu 4°C dan harus digunakan sebelum tanggal expired yang tertera pada box.

Sebelum digunakan untuk uji ELISA, sampel dan reagen terlebih dahulu harus diletakkan pada suhu ruang (18-25°C) (Risto *et al.*, 2013). Kemudian disiapkan reagen-reagen yang akan digunakan untuk ELISA yaitu, 1) *wash buffer* konsentrasi 10x yang diencerkan menjadi 1x (volume *wash buffer* 9 ml dan akuades steril 81 ml), 2) larutan PBS pH 7,4 kosentrasi 10x diencerkan menjadi 1x (volume PBS 100 µl dan akuades steril 900 µl) bahan pembuatan PBS (NaCl = 4 gr, KCl = 0.1 g, Na2HPO4 = 0.72 g, KH2PO4= 0.12g, H2O = 500 ml), 3) *negative control* sebanyak 1.5 ml, 4) MCY-LR *standard* dengan konsentrasi 0.1 (ST1), 0.5 (ST2), 1.0 (ST3), 2.0 (ST4), 5.0 (ST5) ppb (ng/ml) masing-masing sebanyak 1.5 ml, 5) HRP *conjugate* sebanyak 6 ml, 6) *substrate solution* sebanyak 12 ml, dan 7) *stop solution* sebanyak 12 ml.

*Negative control*, larutan standar (ST1-ST5) dan masing-masing sampel hasil preparasi dimasukkan ke dalam *wells* (*microplate*) dengan volume 50 µl, data kode wells ELISA dapat dilihat pada Tabel 4.1. Kemudiantambahkan 50 µl MCY-HRP *enzyme conjugate* pada seluruh

*wells*, tutup *microplate* dengan *aluminium foil*, goyang perlahan menggunakan inkubasi *shaker* selama 30 menit pada suhu ruang (25-37°C) dalam kondisi gelap. Setelah inkubasi selesai, buka *aluminium foil*, buang semua reagen dan cuci menggunakan 1x *washing buffer* 300 µl (lakukan pengulangan sebanyak 3x). Setelah pencucian selesai, keringkan wells dengan cara membuang *washing buffer* pada *tissue*, kemudian menambahkan 100 µl *substrate solution* pada masing-masing *wells*, tutup *microplate* dengan *aluminium foil*, goyang perlahan menggunakan inkubasi *shaker* selama 30 menit pada suhu ruang (25-37°C) dalam kondisi gelap.

Setelah proses inkubasi selesai, akan muncul warna biru pada seluruh larutan pada *wells*, termasuk pada *negative control*. Tambahkan 100 µl *stop solution* pada masing-masing *wells* dan goyang perlahan (total volume pada wells menjadi 200 µl), warna biru pada seluruh larutan di *wells* akan berubah menjadi warna kuning. Kemudian dapat dilakukan pembacaan *microplate* menggunakan ELISA *reader* dengan panjang gelombang 450 nm dalam 3-15 menit setelah penambahan *stop solution*.

Tabel 4.1 Data kode *wells* ELISA

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | BK | ST3 | 1h | 3h | A2 | 6h | 8h | | | | | |
| B | BK | ST3 | 1h | 3h | A2 | 6h | 8h | | | | | |
| C | NC | ST4 | 2i | 4i | 5i | 7i | A3 | | | | | |
| D | NC | ST4 | 2i | 4i | 5i | 7i | A3 | | | | | |
| E | ST1 | ST5 | 2h | 4h | 5h | 7h | A4 | | | | | |
| F | ST1 | ST5 | 2h | 4h | 5h | 7h | A4 | | | | | |
| G | ST2 | 1i | 3i | A1 | 6i | 8i | | | | | | |
| H | ST2 | 1i | 3i | A1 | 6i | 8i | | | | | | |

Keterangan :
BK = Blanko standar, NC = *Negative Control*, ST1 = MCY-LR standar 1; ST2 = MCY-LR standar 2; ST3 = MCY-LR standar 3; ST4 = MCY-LR standar 4; ST5 = MCY-LR standar 5; 1i = Insang udang 1; 1h = Hepatopankreas udang 1; 2i = Insang udang 2; 2h = Hepatopankreas udang 2; 3i = Insang udang 3; 3h = Hepatopankreas udang 3; 4i = Insang udang 4; 4h = Hepatopankreas udang 4; A1 = Air tambak 1; A2 = Air tambak 2; 5i = Insang udang 5; 5h = Hepatopankreas udang 5; 6i = Insang udang 6; 6h = Hepatopankreas udang 6; 7i = Insang udang 7; 7h = Hepatopankreas udang 7; 8i = Insang udang 8; 8h = Hepatopankreas udang 8; A3 = Air tambak 3; A4 = Air tambak 4.

## F. Pembuatan Preparat Histopatologi

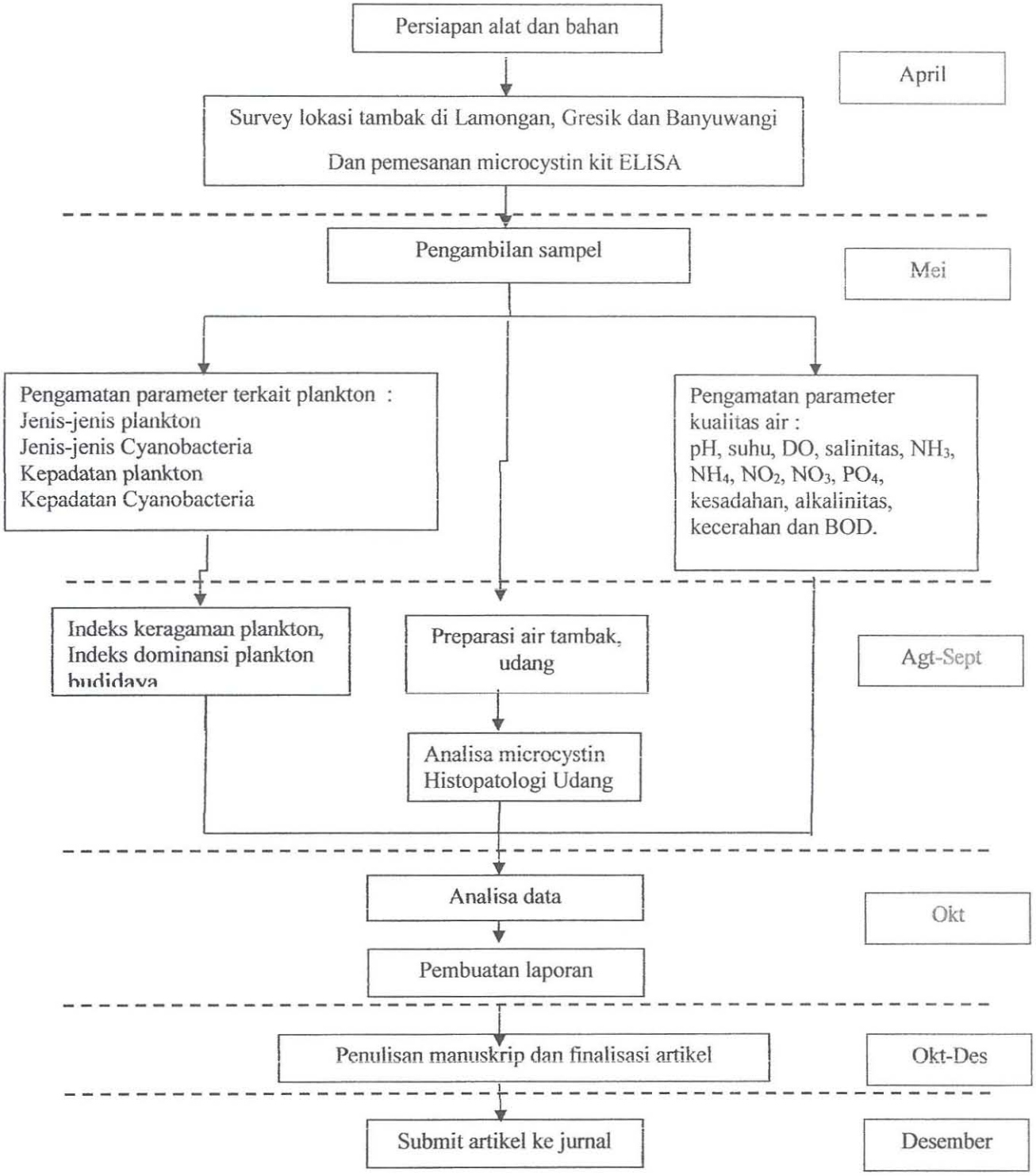
Prosedur pembuatan preparat histopatologis dalam penelitian ini dilakukan berdasarkan prosedur Austin dan Austin (1989) yang meliputi: fiksasi, dehidrasi dan *clearing*, penanaman dalam paraffin (*embedding*), pemotongan, pewarnaan, penutupan dan pengamatan. Organ tubuh udang yang digunakan untuk pembuatan preparat histopatologi dalam penelitian ini yaitu insang dan hepatopankreas.

Insang dan hepatopankreas yang telah diperoleh langsung dimasukkan ke wadah dan direndam dalam larutan fiksatif *Buffer Netral Formalin* (BNF 10 %) (Priosoeryanto *et al.*, 2010), untuk selanjutnya diproses sebagai sediaan histopatologi dengan pewaranaan larutan haematoxylin dan eosin (Sembiring *et al.*, 2013). Spesimen organ (insang dan hepatopankreas) dipotong dengan ukuran 1x1 cm dengan ketebalan 2-3 mm dan diletakkan *cassette.* Selanjutnya dilakukan proses dehidrasi, yaitu proses untuk menarik air dari jaringan dengan merendam organ hasil fiksasi (Priosoeryanto dkk., 2010), selanjutnya didehidrasi dalam larutan alkohol bertingkat mulai dari alcohol 70% , 80%, 90% hingga alkohol absolut 100%.

Tahap selanjutnya adalah tahapan *clearing* dengan cara merendam orgam dalam larutan xylol, diinfiltrasi dengan proses pengisian parafin ke dalam pori-pori jaringan. Kemudian melakukan *blocking,* yaitu proses penanaman spesimen organ ke dalam parafin yang dicetak menjadi blok-blok parafin dalam wadah khusus berupa *tissue cassetë/* blok besi. Selanjutkan *cassete* yang sudah berisi parafin di dinginkan menggunakan *tissue tek* pendingin. Spesimen di keluarkan dari *caseet* dan dipotong dengan ketebalan 3-5µ menggunakan *rotary microtome*.

Potongan-potongan jaringan dimasukkan ke dalam waterbath bersuhu ±50°C, tunggu hingga potongan merenggang kemudian letakkan pada objek *glass*. Preparat yang telah difiksasi pada objek *glass* diwarnai dengan haematoxilin dan eosin menggunakan mesin otomatis *tissu tek* DRS 2000. Setelah tahap pewarnaan selesai, maka dilakukan perekatan menggunakan zat perekat permount dengan entelan, kemudian ditutup dengan *cover glass*, setelah itu preparat siap diamati.

## 4.6 Diagram Alur Penelitian



## 4.7 Analisa Data

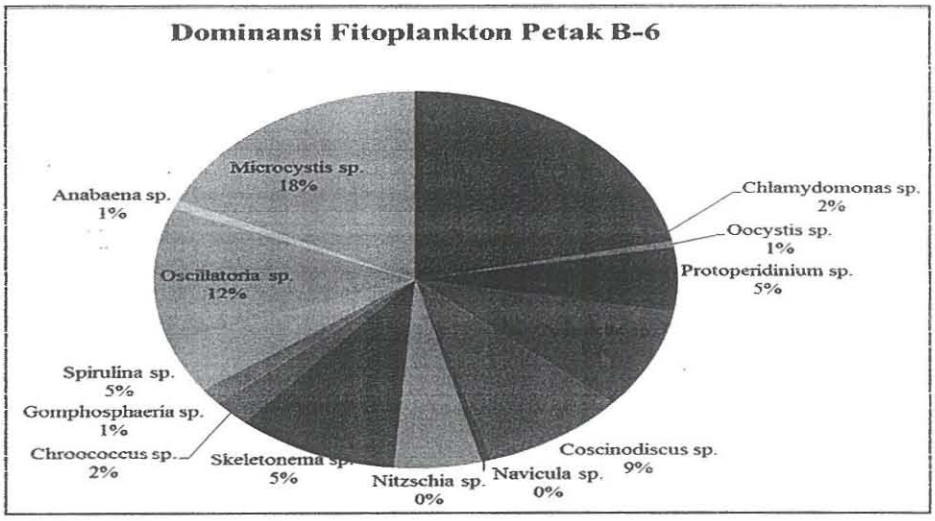
Tahap analisa hasil penelitian dilakukan pada data parameter utama yaitu keragaman dan kepadatan cyanobacteria, kandungan microcystin pada insang dan hepatopankreas udang vaname serta air tambak media budidaya udang tersebut, gambaran histopatologis organ hepatopankreas dan insang udang vaname serta parameter pendukung yaitu keragaman dan kepadatan fitoplankton serta data kualitas air. Analisis dilakukan secara deskriptif dan disajikan berupa gambar, diagram dan tabel untuk kemudian dapat ditar

# V. HASIL DAN PEMBAHASAN

## 5.1 Keragaman dan Dominansi Fitoplankton

Diagram dominansi fitoplankton hasil pengamatan pada petak B-6 disajikan pada Gambar 5.1 dan pada petak B-7 disajikan pada Gambar 5.2, sedangkan tabel komposisi jenis dan kepadatan fitoplankton petak B-6 terdapat pada Lampiran 1 dan petak B-7 pada Lampiran 2.

Hasil pengamatan keragaman jenis fitoplankton pada petak B-6 terdapat 16 genus fitoplankton yang teridentifikasi yaitu *Chlorella* sp., *Chlamydomonas* sp., *Oocystis* sp., *Protoperidinium* sp., *Cyclotella* sp., *Coscinodiscus* sp., *Navicula* sp., *Nitzschia* sp., *Skeletonema* sp., *Streptothecha* sp., *Chroococcus* sp., *Gomphosphaeria* sp., *Spirulina* sp., *Oscillatoria* sp., *Anabaena* sp., dan *Microcystis* sp. Dominansi fitoplankton pada petak B-6 dapat dilihat pada Gambar 5.1 berikut :

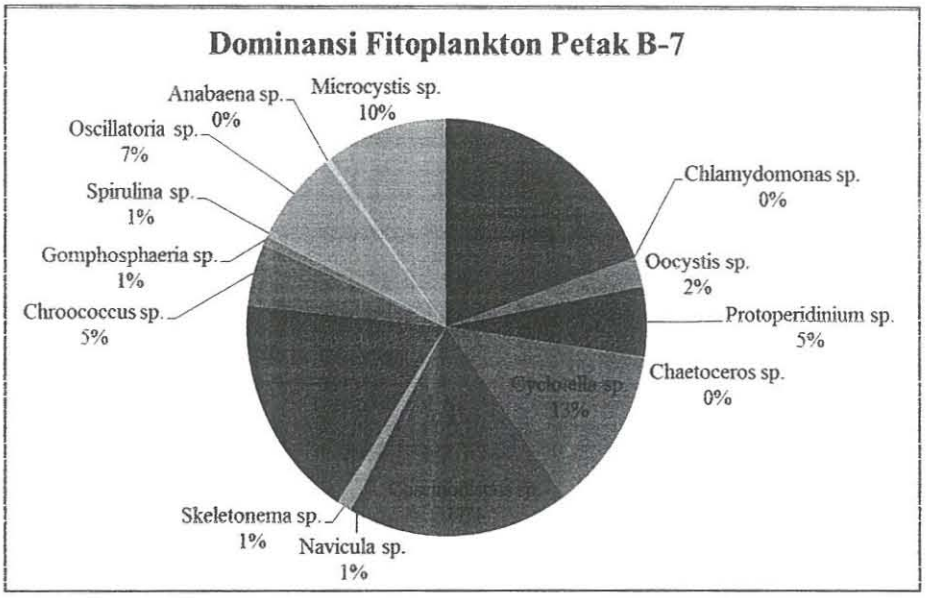



Gambar 5.1 Diagram Dominansi Fitoplankton Petak B-6

Dari diagram tersebut dapat dilihat bahwa petak B-6 di dominasi oleh fitoplankton *Chlorella* sp. (20%) yang merupakan fitoplankton dari kelas Chlorophyceae, *Microcystis* sp. (18%) yang merupakan fitoplankton dari kelas Cyanophyceae dan *Oscillatoria* sp. (12%) yang juga merupakan fitoplankton dari kelas Cyanophyceae.

Hasil pengamatan keragaman jenis fitoplankton pada petak B-7 juga terdapat 16 genus fitoplankton yang teridentifikasi yaitu *Chlorella* sp., *Chlamydomonas* sp., *Oocystis* sp., *Protoperidinium* sp., *Chaetoceros* sp., *Cyclotella* sp., *Coscinodiscus* sp., *Navicula* sp.,

*Skeletonema* sp., *Streptothecha* sp., *Chroococcus* sp., *Gomphosphaeria* sp., *Spirulina* sp., *Oscillatoria* sp., *Anabaena* sp., dan *Microcystis* sp. Dominansi fitoplankton pada petak B-7 dapat dilihat pada Gambar 5.2 berikut :




Gambar 5.2 Diagram Dominansi Fitoplankton Petak B-7

Dari diagram tersebut dapat dilihat bahwa petak B-7 di dominasi oleh fitoplankton *Chlorella* sp. (19%) yang merupakan fitoplankton dari kelas Chlorophyceae, *Streptothecha sp.* dan *Coscinodiscus* sp. (17%) yang merupakan fitoplankton dari kelas Bacillariophyceae dan *Cyclotella* sp. (13%) yang juga merupakan fitoplankton dari kelas Bacillariophyceae.

Hasil pengamatan dominansi cyanobacteria pada petak B-6 dan petak B-7 diperoleh hasil bahwa cyanobacteria lebih mendominasi pada petak B-6, yaitu sebanyak 39%. Nilai dominansi cyanobacteria dan rata-rata konsentrasi microcystin pada sampel yang diamati dapat dilihat pada tabel 5.1 berikut ini:

Tabel 5.1 Dominansi Cyanobacteria dan Nilai rata-rata Konsentrasi Microcystin

| Jenis Cyanobacteria | Dominansi Cyanobacteria | |
|---|---|---|
| | Petak B-6 | Petak B-7 |
| *Microcystis* sp. | 18% | 10% |
| *Anabaena* sp. | 1% | 0% |

| | | |
|---|---|---|
| *Oscillatoria* sp. | 12% | 7% |
| *Spirulina* sp. | 5% | 1% |
| *Gomphosphaeria* sp. | 1% | 1% |
| *Chroococcus* sp. | 2% | 5% |
| Total | 39% | 24% |

| Sampel yang diamati | Nilai rata-rata konsentrasi microcystin ppb (ng/ml) | |
|---|---|---|
| | Petak B-6 | Petak B-7 |
| Air Tambak | 1,7119 | 0,9425 |
| Insang | 1,1809 | 0,0211 |
| Hepatopankreas | 2,8168 | 2,6389 |

### 5.1.1 Jenis-jenis Cyanobacteria yang Tumbuh

Pengamatan terhadap jenis cyanobacteria pada petak B-6 dan B-7 dilakukan setiap hari di waktu pagi (05.00 WIB) dan siang (13.00). Hasil pengamatan cyanobacteria pada petak B-6 dan B-7 ditemukan 6 spesies cyanobacteria, yaitu *Chroococcus* sp., *Gomphosphaeria* sp., *Spirulina* sp., *Oscillatoria* sp., *Anabaena* sp., dan *Microcystis* sp. Komposisi jenis dan kepadatan cyanobacteria pada masing-masing petakan dapat dilihat pada lampiran 1 dan lampiran 2. Jenis cyanobacteria yang ditemukan selama pengamatan didominasi oleh *Microcystis* sp. Alga ini menjadi sangat berbahaya ketika terjadi *blooming* karena menyebabkan dampak yang tidak diinginkan di ekosistem alami perairan (Romanowska-Duda *et al.*, 2002).

Tabel 5.2 Kisaran Nilai Parameter Kualitas Air Selama Penelitian

| Parameter | Kisaran | |
|---|---|---|
| | Petak B-6 | Petak B-7 |
| pH | 7,4-8,6 | 7,4-8,7 |
| Salinitas | 24-25 ppt | 24-25 ppt |
| Alkalinitas | 158-165 mg/l | 125-170 mg/l |
| Suhu | 27-29°C | 27-29°C |
| Kecerahan | 25-45 cm | 20-40 cm |
| DO (*Dissolved Oxygen*) | 3,80-6,50 mg/l | 3,40-8,12 mg/l |

| | | |
|---|---|---|
| Nitrit ($NO_2$) | 2,5-10 mg/l | 0,3-5,0 mg/l |
| Nitrat ($NO_3$) | 3,0-5,0 mg/l | 3,0-5,0 mg/l |
| Amonium ($NH_4^+$) | 0,2-2,3 mg/l | 0,2-3,9 mg/l |
| Fosfat ($PO_4$) | 0,25-1,25 mg/l | 0,3-1,75 mg/l |
| N/P Rasio | 6,7-28,9 | 2,4-28,4 |

## 5.2 Konsentrasi Microcystin

Konsentrasi microcystin pada seluruh sampel (organ insang, hepatopankreas dan air tambak media budidaya udang) dapat diketahui melalui perhitungan menggunakan rumus persamaan linear garis regresi pada Microsoft Excel, dengan memasukkan nilai konsentrasi MCY-LR standar sebagai nilai x dan nilai rata-rata absorbansi MCY-LR standar pada pembacaan Elisa *reader* sebagai nilai y. Hasil nilai absorbansi MCY-LR standar dan seluruh sampel menggunakan Elisa *reader* dapat dilihat pada lampiran 4. Konsentrasi microcystin pada sampel (x) dihitung menggunakan rumus persamaan linear garis regresi (y= -0,0991x + 0,9924) dengan nilai rata-rata absorbansi sampel sebagai (y) maka akan diketahui nilai konsentrasi microcystin (x).

### 5.2.1 Konsentrasi Microcystin dalam Air Tambak Budidaya Intensif Udang Vaname

Konsentrasi microcystin pada sampel air tambak media budidaya intensif udang vaname (*Litopenaeus vannamei*) menunjukkan nilai konsentrasi yang berbeda antara petak B-6 dengan petak B-7. Air tambak media budidaya udang vaname pada petak B-6 menunjukkan nilai konsentasi microcystin lebih tinggi daripada petak B-7. Hasil konsentrasi microcystin pada air tambak media budidaya intensif udang vaname selengkapnya disajikan pada Tabel 5.3 berikut :

Tabel 5.3 Data konsentrasi microcystin dalam air budidaya intensif udang vaname

| Petak | Waktu (WIB) | Konsentrasi Microcystin ppb (ng/ml) |
|---|---|---|
| B-6 | 05.00 | 2,113 |
| | 13.00 | 1,3108 |

| | | |
|---|---|---|
| B-7 | 05.00 | 1,3108 |
| | 13.00 | 0,5742 |

### 5.2.2 Konsentrasi Microcystin dalam Organ Insang dan Hepatopankreas Udang Vaname

Konsentrasi microcystin dalam organ insang dan hepatopankreas sampel udang vaname dari petak B-6 dan B-7 menunjukkan nilai konsentrasi yang berbeda-beda. Organ insang udang vaname pada petak B-6 menunjukkan nilai konsentasi microcystin lebih tinggi daripada petak B-7. Hasil konsentrasi microcystin selengkapnya disajikan pada Tabel 5.4 berikut :

Tabel 5.4 Data konsentrasi microcystin dalam organ insang dan hepatopankreas udang vaname

| Petak | Microcystin Insang ppb (ng/ml) | Microcystin Hepatopankreas ppb (ng/ml) |
|---|---|---|
| B-6 | 0,6095 | 3,6973 |
| B-7 | 0,01 | 2,2896 |
| B-6 | 0,6852 | 1,5378 |
| B-7 | 0,01 | 2,1635 |
| B-6 | 1,3108 | 3,9596 |
| B-7 | 0,01 | 2,1079 |
| B-6 | 2,1181 | 2,0726 |
| B-7 | 0,0545 | 3,9949 |

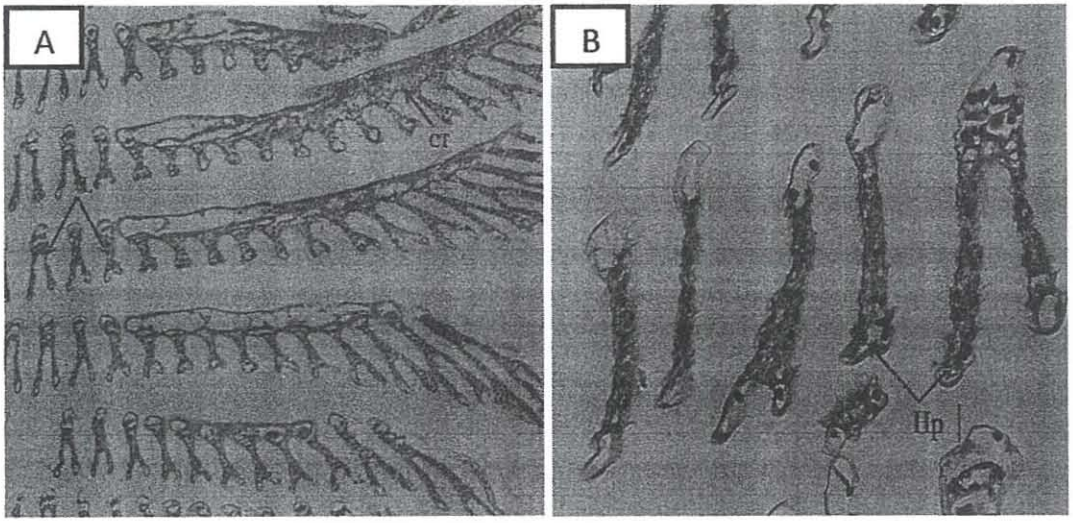
## 5.3 Histopatologi Organ Udang Vaname

Pengamatan preparat histopatologi dari organ sampel dapat membantu untuk mengetahui perubahan histopatologi yang terjadi pada organ tersebut secara lebih jelas dan tepat (Mahasri *et al.*, 2008). Identifikasi histopatologi pada penelitian ini menggunakan sampel organ insang dan hepatopankreas udang vaname (*Litopenaeus vannamei*). Pengambilan sampel dilakukan saat udang vaname berumur tiga bulan (DOC 94) dengan panjang 15-18 cm dan berat 5-6 gram. Pengamatan preparat histopatologi organ insang dan hepatopankreas udang vaname pada tambak budidaya intensif yang berlokasi di Kabupaten

Banyuwangi ini menunjukkan adanya perubahan mikroskopis pada organ insang dan hepatopankreas.

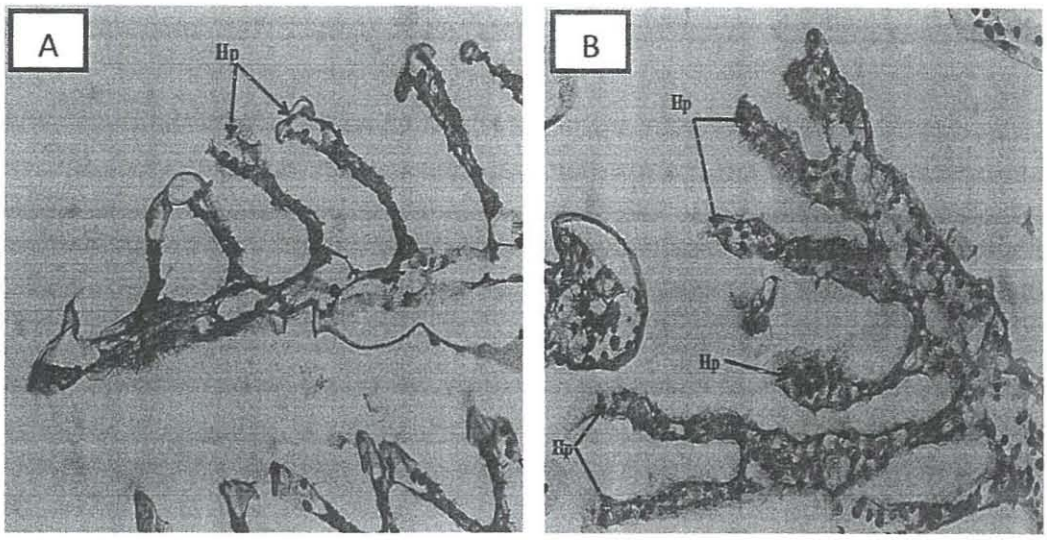
### 5.3.1 Histopatologi Organ Insang Udang Vaname

Hasil pengamatan preparat histopatologi sampel udang vaname organ insang mengalami hiperplasia yang ditemukan di bagian dasar lamella. Lamella adalah tempat pertukaran oksigen, jika terjadi kerusakan pada lamela tersebut, akibatnya peredaran darah akan terganggu dan terjadi pembendungan darah. Semakin lama, kerusakan ini akan menyebabkan gangguan sirkulasi yang dapat mengakibatkan suplai oksigen berkurang. Pada akhirnya, akan terjadi efek letal karena terganggunya sistem pernafasan (Rizki *et al.*, 2015). Gambaran histopatologi insang udang vaname pada petak B-6 disajikan pada gambar 5.3 dan histopatologi insang udang vaname pada petak B-7 disajikan pada gambar 5.4 berikut :




Gambar 5.3 Histopatologi Insang Udang Vaname Petak B-6

a. L = Lamellae; CT = Connective tissues; (HE, 100x)
b. Hp = Hiperplasia (HE, 200x)



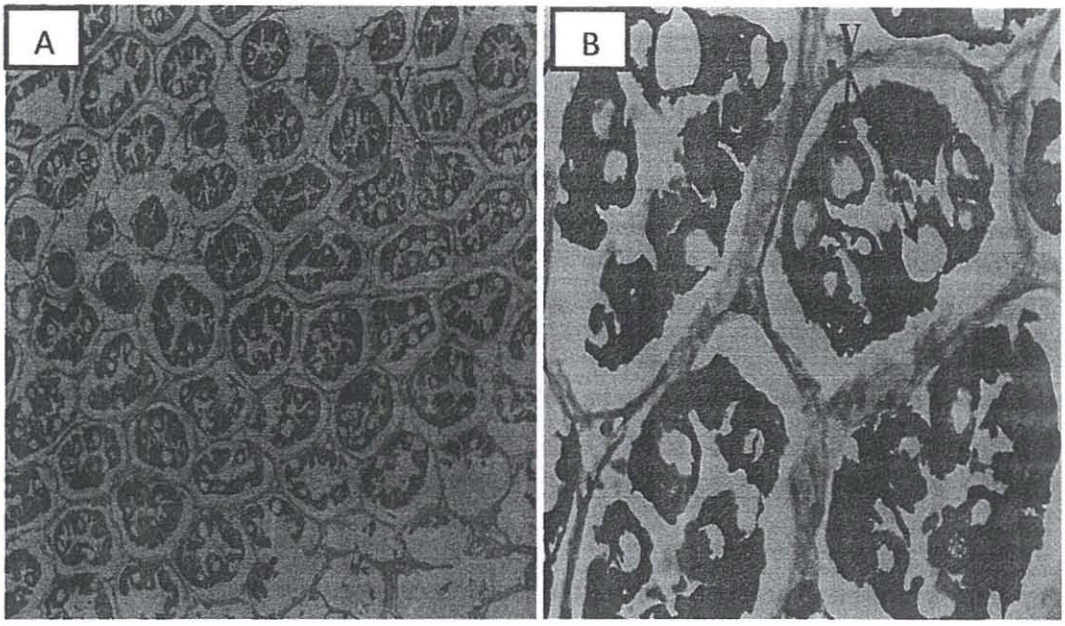

Gambar 5.4 Histopatologi Insang Udang Vaname Petak B-7

a. Hp = Hiperplasia (HE, 200x)
b. Hp = Hiperplasia (HE, 200x)

Hiperplasia adalah pembentukan jaringan secara berlebihan karena bertambahnya jumlah sel (Laksmana, 2003). Lamella yang mengalami hiperplasia ditandai dengan bertambahnya jumlah sel di lamella, lamella menjadi tebal (Soegianto *et al*., 2004).

### 5.3.2 Histopatologi Organ Hepatopankreas Udang Vaname

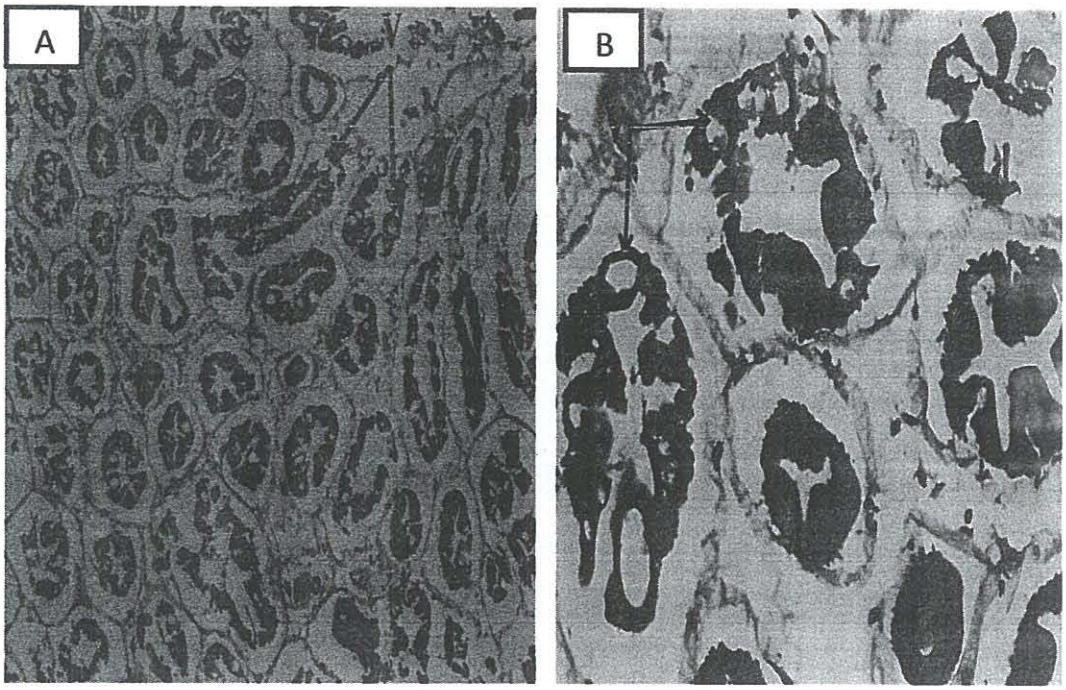
Hasil pengamatan preparat histopatologi sampel organ hepatopankreas udang vaname mengalami vakuolisasi. Histopatologi hepatopankreas udang vaname pada petak B-6 disajikan pada gambar 5.5 dan histopatologi hepatopankreas udang vaname pada petak B-7 disajikan pada gambar 5.6 berikut :




Gambar 5.5 Histopatologi Hepatopankreas Udang Vaname Petak B-6

a. V = Vakuolisasi (HE, 100x)
b. V = Vakuolisasi (HE, 400x)

Vakuolisasi adalah pembentukan ruang di dalam sel yang berisi lemak akibat dari degenerasi sel yang ditandai dengan munculnya vakuola-vakuola pada tubulus hepatopankreas (Budi, 2007). Vakuolisasi dapat terjadi karena adanya penimbunan lemak pada tubulus hepatopankreas dan gangguan pada proses oksidasi yang akan menyebabkan terjadinya penimbunan lemak di hepatopankreas. (Price and Wilson, 1989). Kerusakan hepatopankreas ini menyebabkan pembusukan sel dan lisis sel, sehingga mengganggu proses metabolisme tubuh udang (Yanto, 2006).




Gambar 5.6 Histopatologi Hepatopankreas Udang Vaname Petak B-7

a. V = Vakuolisasi (HE, 100x)
b. V = Vakuolisasi (HE, 400x)

## 5.4 Pembahasan

Cyanobacteria atau alga hijau biru merupakan kelompok alga prokariotik (Graham and Wilcox, 2000). Organisme tersebut memiliki peran sebagai produsen dan penghasil senyawa nitrogen di perairan (Adnan, 1993). Beberapa Cyanobacteria juga diketahui dapat memproduksi toksin (racun) (Hoek *et al.*, 1995). Hasil pengamatan fitoplankton pada petak B-6 di dominasi oleh *Chlorella* sp. (20%) yang merupakan fitoplankton dari kelas Chlorophyceae, *Microcystis* sp. (18%) yang merupakan fitoplankton dari kelas Cyanophyceae dan *Oscillatoria* sp. (12%) yang juga merupakan fitoplankton dari kelas Cyanophyceae. Hal ini menunjukkan bahwa petak B-6 memiliki nilai dominansi cyanobacteria yang tinggi. Jenis cyanobacteria yang mendominasi adalah *Microcystis* sp dan *Oscillatoria* sp. Peningkatan dan penurunan keragaman dan dominansi dipengaruhi oleh banyak faktor seperti kualitas air, unsur hara perairan dan faktor lingkungan (Goldman dan Horne, 1983).

Hasil pengamatan fitoplankton pada petak B-7 di dominasi oleh Chlorella sp. (19%) yang merupakan fitoplankton dari kelas Chlorophyceae, *Streptothecha sp.* dan *Coscinodiscus* sp. (17%) yang merupakan fitoplankton dari kelas Bacillariophyceae dan *Cyclotella* sp. (13%) yang juga merupakan fitoplankton dari kelas Bacillariophyceae. Hal ini menunjukkan bahwa petak B-7 tidak memiliki nilai dominansi cyanobacteria yang tinggi. Petak B-7 di dominasi oleh fitoplankton dari kelas Chlorophyceae dan Bacillariophyceae.

Pertumbuhan cyanobacteria dipengaruhi oleh faktor fisik, kimia, dan biologis. *Blooming* Cyanobacteria terjadi pada pasokan air yang mengandung cukup kandungan nutrisi anorganik penting seperti nitrogen dan fosfor, suhu air yang umumnya berkisar antara 15 – 30°C , serta tingkat pH antara 6 – 9 (WHO, 2003). Variasi pH dapat mempengaruhi metabolisme dan pertumbuhan fitoplankton melalui beberapa hal, antara lain mengubah keseimbangan dari karbon organik, mengubah ketersediaan nutrien dan dapat mempengaruhi fisiologis sel. Kisaran pH air yang layak untuk pertumbuhan fitoplankton adalah 6,5-8,5 (Boyd, 1990). Hasil pengukuran parameter kualitas air pada penelitian ini menunjukkan kisaran nilai pH antara 7,4-8,6 pada petak B-6 dan 7,4-8,7 pada petak B-7. Kisaran nilai tersebut sesuai untuk pertumbuhan cyanobacteria (fitoplankton). Menurut Xu *et al.* (2010) perairan dengan pH antara 6–9 merupakan perairan dengan kesuburan yang tinggi dan tergolong produktif karena pada kisaran pH tersebut dapat mendorong proses pembongkaran bahan organik yang ada dalam perairan oleh bakteri aerob menjadi mineral-mineral yang dapat dimanfaatkan oleh fitoplankton untuk pertumbuhan.

Fitoplankton memiliki faktor pembatas pertumbuhan, diantaranya ialah salinitas. Fluktuasi salinitas secara langsung menyebabkan perubahan tekanan osmosis di dalam sel mikroalga. Salinitas yang tinggi atau rendah dapat menyebabkan tekanan osmosis di dalam sel, sehingga aktivitas sel menjadi terganggu. Hal ini dapat mempengaruhi pH sitoplasma sel dan menurunkan kegiatan enzim di dalam sel. Salinitas optimum bagi pertumbuhan mikroalga antara 25-35 ppt (Tjahjo *et al.*, 2002). Hasil pengukuran salinitas pada penelitian ini menunjukkan nilai yang optimal untuk pertumbuhan fitoplankton, yaitu berkisar antara 24-25 ppt pada kedua petak.

Hasil pengukuran alkalinitas pada penelitian ini menunjukkan nilai yang berkisar antara 158-165 mg/l pada petak B-6 dan 125-170 mg/l pada petak B-7. Nilai tersebut kurang optimal untuk kegiatan budidaya, karena melebihi 150 mg/l. Alkalinitas sangat dibutuhkan di dalam perairan karena alkalinitas merupakan kemampuan air dalam menetralisir asam perairan (*Buffer* pH) (Santhosh and Singh, 2007). Apabila alkalinitas di dalam perairan tinggi maka fluktuasi pH yang tinggi tidak akan terjadi. Sebaliknya apabila alkalinitas didalam perairan rendah maka fluktuasi pH akan tinggi yang dapat mengakibatkan dampak buruk baik secara langsung maupun tidak langsung terhadap organisme perairan (Rukminasari dkk., 2014).

Suhu juga sangat berpengaruh terhadap pertumbuhan, jika suhu terlalu rendah maka akan menghambat pertumbuhan dan jika suhu terlalu tinggi dapat mematikan pada fitoplankton (Lavens and Sorgeloos, 1996). Hasil pengukuran suhu selama penelitian berkisar antara 27-29°C pada kedua petak. Suhu optimum untuk produksi toksin pada cyanobacteria adalah antara 20 dan 25°C, yang menunjukkan bahwa cyanobacteria paling beracun dengan cuaca hangat dan di daerah-daerah dengan iklim hangat (Watanabe & Oishi, 1985). Suhu air di permukaan dipengaruhi oleh kondisi meteorologi seperti : curah hujan, penguapan, kelembaban udara, suhu udara, kecepatan angin, dan intensitas radiasi matahari (Nontji, 2007).

Hasil pengukuran kandungan oksigen terlarut (DO) pada petak B-6 pengamatan pagi hari berkisar antara 3,80-4,60 mg/l. Sedangkan pada pengamatan siang hari berkisar antara 6,0-6,50 mg/l. Kandungan DO pada petak B-7 di pengamatan pagi hari berkisar antara 3,40-4,47 mg/l. Sedangkan pada pengamatan siang hari berkisar antara 7,80-8,12 mg/l. Berdasarkan hasil diatas diketahui bahwa kandungan oksigen terlarut di tambak intensif udang vaname dapat dikatakan optimal untuk kegiatan budidaya khususnya untuk plankton. Hal ini sesuai dengan pendapat Wijayanti (2011) yang menyatakan bahwa plankton dapat hidup baik pada kandungan oksigen terlarut lebih dari 3 mg/1.

Kebutuhan kandungan dan jenis nutrien *Algae* sangat tergantung pada kelas atau jenisnya pada habitat tersebut. Nutrien yang paling penting untuk pertumbuhan *Algae* antara lain adalah nitrogen (Tubalawony, 2007). Nitrogen juga merupakan bahan penting penyusun asam amino, amida, nukleotida, dan nukleo protein, serta essensial untuk pembelahan sel sehingga nitrogen penting penting untuk pertumbuhan. *Blue green algae* juga membutuhkan

fosfat untuk pertumbuhan optimalnya, BGA tidak akan tumbuh pada kondisi ketersediaan P yang terbatas (Roger and Kulasooriya, 1980). Namun, keberadaan fosfat secara berlebihan yang disertai dengan keberadaan nitrogen fosfat dan nitrogen seperti amoniak, nitrit dan nitrat yang terdapat di tambak dapat menstimulir ledakkan pertumbuhan *algae* di perairan (*algae bloom*). Senyawa tersebut bersifat metabolitoksik dan sangat berbahaya bagi perikanan tambak (Hendrawati *et al.*, 2008).

Senyawa amonium, nitrit dan nitrat merupakan bentuk lain dari nitrogen anorganik. Effendi (2003) menyatakan bahwa nitrogen anorganik terdiri dari ammonia ($NH_3$), amonium ($NH_4$), nitrit ($NO_2^-$), dan nitrogen ($N_2$). Hasil pengukuran nilai amonium pada petak B-6 selama penelitian berkisar antara 0,2-2,3 mg/l. Sedangkan pada petak B-7 berkisar antara 0,2-3,9 mg/l. Nilai tersebut kurang optimal untuk kegiatan budidaya, karena melebihi 1 mg/l. Menurut Philminaq (2006), kisaran amonium yang dapat ditolerir dalam kegiatan budidaya adalah <1mg/l.

Hasil pengukuran nilai nitrit pada petak B-6 berkisar antara 2,5-10 mg/l. Sedangkan pada petak B-7 berkisar antara 0,3-5,0 mg/l. Sedangkan nilai nitrat pada petak B-6 berkisar antara 3,0-5,0 mg/l. Hasil yang sama pada petak B-7 yaitu berkisar antara 3,0-5,0 mg/l. Menurut Bhatnagar and Devi (2013), kisaran optimum nitrit untuk budidaya yaitu <0.02 mg/l sedangkan kisaran optimum nitrat untuk budidaya yaitu 0.1-4.5 mg/l. Senyawa nitrit yang berlebih di tambak akan menyebabkan menurunnya kemampuan darah udang untuk mengikat $O_2$, karena nitrit akan bereaksi lebih kuat dengan hemoglobin yang mengakibatkan tingkat kematian udang tinggi. Selain itu, tingginya senyawa amonia dan nitrit di tambak juga akan menganggu proses pengeluaran senyawa amonia dan nitrit yang ada dalam tubuh udang, sehingga akan terakumulasi di dalam tubuh udang (Trobos, 2007). Hasil pengukuran fosfat pada penelitian ini menunjukkan nilai fosfat pada petak B-6 berkisar antara 0,25-1,25 mg/l. Sedangkan pada petak B-7 berkisar antara 0,3-1,75 mg/l. Kisaran nilai tersebut optimal untuk kegiatan budidaya. Menurut Haliman dan Adijaya (2005), kisaran nilai fosfat yang optimal untuk budidaya adalah 1-3 mg/l.

Faktor pembatas untuk pertumbuhan mikroalga adalah N dan P (Dallaire, *et al*,. 2007). Nilai N/P rasio pada petak B-6 selama penelitian berkisar antara 6,7-28,9. Sedangkan pada

petak B-7 berkisar antara 2,4-28,4. Nilai N/P rasio tertinggi terjadi pada petak B-6 yaitu sebesar 28,9 dan nilai N/P rasio terendah terjadi pada petak B-7 yaitu sebesar 2,4. Pada akhir penelitian, pada petak B-6 diperoleh nilai N/P rasio 8,2. Sedangkan pada petak B-7 diperoleh nilai N/P rasio sebesar 7,9. Nilai N/P rasio ini tergolong rendah, rendahnya nilai N/P rasio tersebut (<10), menyebabkan plankton jenis *Blue Green Algae*/Cyanobacteria menjadi dominan. Menurut Aiyushirota (2009) distribusi plankton seperti *Blue Green Algae*/Cyanobacteria dan dinoflagellata ditentukan oleh proporsi nilai nitrogen terhadap fosfor, pada lingkungan eutrofik dengan perbandingan total ppm N dibagi total ppm P apabila di bawah 10 (N:P rasio<10) maka didominasi dengan pertumbuhan BGA atau *Blue Green Algae*. Pada nilai N/P rasio yang rendah maka konsentrasi N relatif rendah dengan konsentrasi P relatif tinggi, sehingga diatom, plankton dan bakteri pertumbuhannya terbatas karena tidak memperoleh tambahan asupan N lainnya. Sementara *Blue Green Algae*/Cyanobacteria dan beberapa spesies Dinoflagellata dapat mengabsorpsi N langsung dari udara, sehingga pertumbuhannya tidak terbatasi.

Spesies-spesies Cyanobacteria yang ditemukan pada penelitian ini yaitu *Chroococcus* sp., *Gomphosphaeria* sp., *Spirulina* sp., *Oscillatoria* sp., *Anabaena* sp., dan *Microcystis* sp. Spesies-spesies tersebut diketahui dapat memproduksi toksin microcystin. Microcystin adalah golongan dari racun peptida siklik yang diproduksi oleh cyanobacteria air payau dan air tawar dari genera *Anabaena*, *Anabaenopsis*, *Chroococcus*, *Microcystis*, *Nostoc*, *Planktothrix* dan *Oscillatoria* (Chorus, 2001; Prihantini *et al.*, 2006; Pearson *et al.*, 2010). Toksin ini adalah produk metabolit sekunder yang terakumulasi dalam sitoplasma pada situasi tertentu (Paerl dan Millie, 1996). Microcystin stabil dalam air dan bersifat hepatotoksik (Romanowska-Duda *et al.*, 2002).

Berdasarkan hasil pengukuran kosentrasi microcystin pada organ insang dan hepatopankreas udang vaname serta air tambak media budidaya udang, ketiga macam sampel tersebut menunjukkan hasil konsentrasi microcystin yang berbeda-beda. Konsentrasi microcystin tertinggi diperoleh dari sampel hepatopankreas udang vaname, yaitu sebanyak 3,9949 ppb (ng/mL) dengan rata-rata 2,7279 ppb (ng/mL). Hal ini sesuai dengan pernyataan

USEPA (2006) yaitu kebanyakan microcystin merupakan hepatotoksin/racun hati dan hati merupakan target utama microcystin (WHO, 2003).

Kosentrasi microcystin terendah diperoleh dari sampel insang udang vaname, yaitu sebanyak 0,01 ppb (ng/mL) dengan rata-rata 0,0211 ppb (ng/mL). Rendahnya konsentrasi microcystin pada sampel insang udang vaname diduga karena paparan toksin pada insang terjadi secara pasif, yaitu melalui kontak dengan epitel insang (Malbrouck and Kestemont, 2006).

Sedangkan pada sampel air tambak media budidaya udang vaname diperoleh hasil konsentrasi microcystin terendah yaitu 0,5742 ppb (ng/mL) dan konsentrasi microcystin tertinggi sebanyak 2,1130 ppb (ng/mL) dengan rata-rata 1,2121 ppb (ng/mL). Konsentrasi microcystin pada sampel air tambak tersebut cukup tinggi, hal ini dikarenakan toksin cyanobacteria (microcystin) dapat dirilis ke dalam air selama mereka mengalami penuaan hingga lisis, toksin tersebut dapat larut dalam air (Drobac *et al.*, 2016).

Di antara cyanotoxin, microcystin dengan promotor hepatotoksik yang juga dapat menyebabkan tumor hati dianggap sebagai salah satu kelompok toksin yang paling berbahaya (Li *et al.*, 2017). Paparan microcystin juga terjadi secara pasif, yaitu melalui kontak langsung epitel insang dengan air sekitarnya yang mengandung toksin tersebut (Malbrouck and Kestemont, 2006). Oleh karena itu pada penelitian ini dilakukan pembuatan preparat histopatologi organ insang dan hepatopankreas udang vaname (*Litopenaeus vannamei*) agar dapat di amati secara mikroskopis.

Berdasarkan hasil pengamatan preparat histopatologi organ insang udang vaname tampak adanya hiperplasia. Beberapa kejadian patologis yang banyak ditemukan pada pengamatan preparat histopatologi organ insang udang vaname yaitu adanya hiperplasia. Mulyani *et al.,* (2014), menyatakan bahwa hiperplasia dapat terjadi akibat stimuli kimia dari polutan-polutan, pencemaran lingkungan, infeksi parasit dan bakteri. Hiperplasia selain akan menekan kapiler pembuluh darah pada sel juga akan memerlukan peningkatan suplai darah ke jaringan yang baru terbentuk. Pada kondisi kronis sekali keadaan sel sudah tidak berbentuk normal lagi tetapi akan saling menempel (Hibiya, 1995).

Pada hasil pengamatan preparat histopatologi organ hepatopankreas udang vaname tampak adanya vakuolisasi, yaitu pembentukan ruang didalam sel yang berisi lemak akibat dari degenerasi sel yang ditandai dengan munculnya vakuola-vakuola pada tubulus hepatopankreas. Vakuolisasi ditandai dengan sel-sel epitel tubulus yang terlihat dibawah mikroskop kehilangan isi selnya atau kosong (Soegianto, et al., 2004).

Insang berperan pada proses respirasi, keseimbangan asam basa, regulasi ionik dan osmotik karena adanya jaringan *epithelium branchial* yang menjadi tempat berlangsungnya transport aktif ion-ion penting antara organisme dan lingkungan (Soegianto *et al.*, 1999), serta memainkan peran penting dalam toksikologi krustasea (Morales-Covarrubias *et al.*, 2016). Sedangkan hepatopankreas adalah organ terpenting pada udang, karena organ tersebut berfungsi seperti hati dan pankreas pada mamalia (Soegianto *et al.*, 1999). Tingginya kerusakan pada struktur insang dan hepatopankreas, akan berpengaruh pada proses metabolisme enzim dan osmoregulasi pada udang. Selain itu kerusakan pada sel yang disebabkan oleh keracunan atau faktor lain, sebagai contoh yaitu kondisi stres, bisa meningkatkan sensitivitas terhadap infeksi virus dan bakteri (Snieszko, 1974). Hal ini dapat dengan cepat meningkatkan tingkat risiko kematian pada udang (Soegianto *et al.*, 2004).

Hal ini sesuai dalam dengan pernyataan Sousa dan Petriella (2007) hepatopankreas sangat sensitif terhadap pengaruh pencemaran, sehingga organ ini sering digunakan untuk mengetahui efek dari berbagai toksikan. Perubahan histopatologis dapat memberikan informasi terhadap tingkat stres, kerentanan dan adaptiptasi kemampuan organisme menghadapi stress, seperti halnya pada organ hepatopankreas udang vaname.

## 5.5 Luaran yang Dicapai

Luaran penelitian ini terangkum pada tabel berikut ini

| No | Jenis Luaran | Nama mahasiswa / Event | Keterangan |
|---|---|---|---|
| 1. | Skripsi mahasiswa S1 | Doni Husein | lulus |
| | | Dzaki Zulian | lulus |
| | | Rama Satrya Putra | lulus |
| | | Tia Rahma | lulus |
| | | Aldo Lovely Arief | lulus |
| 2. | Thesis mahasiswa Pascasarjana Universitas Airlangga | Fitri Annisa | lulus |
| | | M. Riza Noor T | Sedang penelitian lanjutan |
| 3. | Oral Presentation dan Artikel pada Proceeding International Conference on Fisheries and Marine (Incofim 2018, bekerjasama dg IOP, terindex Scopus) (FPK Unair, 6 Oktober 2018) | Dynamic Ratio Correlation N:P toward Diatom Abundance in The Intensive System Vannamei Shrimp Pond | Under revision |
| | | Dynamic Ratio Correlation N:P toward Abundance of Blue Green Algae in Intensive System of Vannamei Shrimp Pond | Under revision |
| | | Dynamic Ratio Correlation N:P toward Phytoplankton Explosion in Intensive System of Vannamei Shrimp Pond | Under revision |
| 4 | Oral Presentation pd IFS 2018 (International Fisheries Symposium) di Faculty of Science and Technology, Prince Songkla University., Thailand,18-21 Nop 2018 + Artikel u Publikasi | Microcystin Toxin Production of Several Blue Green Algae Species from Banyuwangi, Indonesia, in Different Salinity Culture | Menunggu pengumuman dari Panitia untuk masuk pada jurnal atau proceeding + Draft Artikel |
| 5 | Artikel untuk Publikasi pada Jurnal terindex Scopus | Micocystin Concentration in Vannamei (*Litopenaeus vannamei*) Intensive Ponds in Banyuwangi, East Java | Draft, rencana submit sblm 29-12-2018 pada Indiana Veterinay Journal atau Iranian Journal of Fisheries Sciences |
| 6 | Nara sumber pd Seminar Nasional Shrim Club Indonesia cabang Banyuwangi | Microcystin di Perairan Jawa Timur | Foto kegiatan |
| 7. | Oral Presentation pada Academic Assimilation di UMT, Malaysia, 2018 | Microcystis as harmfull Algae | Invitation Letter, Sertifikat sbg Pembicara dan Foto Kegiatan |

# BAB 6. RENCANA TAHAPAN BERIKUTNYA

Rencana tahap berikutnya adalah eksplorasi kandungan microcystin pada tambak tawar di wilayah Gresik dan Lamongan (Saat ini penelitian sedang berjalan). Hal ini perlu dilakukan mengingat Cyanobacteria sebenarnya merupakan plankton perairan tawar. Sedangkan tambak-tambak tawar di Jawa Timur, umumnya merupakan tambak tradisional yang tergenang (resirkulasi air minimal), sehingga penumpukan bahan organic tinggi. Hal ini sangat mendukung terjadinya blooming Cyanobacteria terutama Microcystis penghasil racun microcystin. Gresik dan Lamongan merupakan salah satu sentra tambak tradisional terbesar di Jawa Timur. Sehingga pemetaan potensi keberadaan racun microcystin perlu diketahui untuk mendukung upaya pengelolaan tambak agar keamanan pangan dapat terjaga.

# BAB 7. KESIMPULAN DAN SARAN

## 7.1 Kesimpulan

Berdasarkan hasil penelitian yang telah dilakukan, maka kesimpulan dari penelitian ini adalah :

1. Jenis cyanobacteria yang ditemukan pada penelitian ini adalah *Microcystis* sp., *Anabaena* sp., *Oscillatoria* sp., *Spirulina* sp., *Gomphosphaeria* sp. dan *Chroococcus* sp.
2. Semakin rendah rasio N:P perairan, keragaman Cyanobacteria semakin rendah, atau terjadi dominansi oleh salah satu jenis spesies Cyanobacteria.
3. Rasio N;P perairan diusahakan lebih dari 15, untuk menghindari blooming Cyanobacteria
4. Dominansi cyanobacteria mempengaruhi konsentrasi toksin microcystin pada sampel yang diamati di petakan yang berbeda, yaitu organ insang dan hepatopankreas udang vaname (*Litopenaeus vannamei*) serta air media budidaya udang.
5. Akumulasi toksin microcystin pada organ insang dan hepatopankreas udang vaname (*Litopenaeus vannamei*) juga menunjukkan adanya perubahan histopatologi pada pengamatan preparat histopatologi organ insang dan hepatopankreas.

## 7.2 Saran

Ditinjau dari hasil penelitian bahwa toksin microcystin terakumulasi dalam organ insang, hepatopankreas dan air media budidaya udang vaname (*Litopenaeus vannamei*), maka saran dari penelitian ini adalah diperlukan penelitian lebih lanjut mengenai konsentrasi toksin microcystin secara reguler pada otot/daging udang untuk mengevaluasi dampak paparan microcystin dan menjamin keamanan pangan.

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**Lampiran 1. Personalila Tenaga Pelaksana beserta Kualifikasinya**

**Personalila Tenaga Pelaksana beserta Kualifikasinya**

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Lampiran 2. Certificate as Presenter pada Incofim 2018 dan Artikel pada Proceeding International Conference on Fisheries and Marine (Incofim 2018) (Under Revision)

Artikel 1 :
Dynamic Ratio Correlation N:P toward Diatom Abundance in The Intensive System Vannamei Shrimp Pond

Artikel 2 :

Dynamic Ratio Correlation N:P toward Abundance of Blue Green Algae in Intensive System of Vannamei Shrimp Pond

Artikel 3 :

Dynamic Ratio Correlation N:P toward Phytoplankton Explosion in Intensive System of Vannamei Shrimp Pond


1st INTERNATIONAL CONFERENCE ON FISHERIES AND MARINE SCIENCES

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October 06th 2018

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# Dynamic Ratio Correlation N:P toward Phytoplankton Explosion in Intensive System of Vannamei Shrimp Pond

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**Abstract.** Phytoplankton which a supporting microorganism within the activities of vannamei shrimp cultivation, plays a role as natural weft as the characteristic of phytoplankton organism is foundation of food chain in water, beside in the activities of vannamei shrimp intensive cultivation phytoplankton also useful to control color and clarity of the water, increase dissolved oxygen (DO) levels of water, control the growth of weed in bottom of the pond, and absorbed the abundance of organic compounds in the water such as ammonium, nitric and nitrate, the nutrient needed to grow phytoplankton. This research aimed to find out the influence of ratio dynamic N:P toward the abundance of phytoplankton in intensive system shrimp pond. The main parameters to be observed are ammonium, nitric, nitrate and phosphate, as well as abundance of phytoplankton and will be held in laboratory of PT Surya Windu Kartika Banyuwangi, the measurement is also done toward supporting parameters of pH, clarity and salinity. The result of data analysis and study revealed that dynamic composition of ammonium, nitric, nitrate and phosphate is influencing dynamic composition of phytoplankton in the water. The increasing of nitric levels affects to increase composition of Bacillariapyceae and decrease abundant composition of cyanophyceae, increase level of ammonium in the water cause increase abundant composition of cyanophyceae and decrease abundant composition of Bacillariapyceae, and dynamic PO4 influence dynamic abundant composition of phytoplankton classified as Bacillariapyceae and cyanophyceae

Comment [A1]: The abstract more than 200 words

## 1. Introduction

Phytoplankton is a supporting microorganism in the activities of Vannamei shrimp cultivation. Phytoplankton is important as natural feed due to its characteristic of the organism as foundation of food chain in the aquatic system [1], besides in the activities of intensive shrimp cultivation

phytoplankton is also important to control watercolor and turbidity, increase level of dissolved oxygen (DO) in the water, restrain growth of the moss in the bottom of pond, and also to absorb harmful organic compounds for shrimp like ammonia, nitrite and nitrate, which are necessary nutrients for growing phytoplankton [2].

Diversity and abundance of phytoplankton are useful as stability parameter in the nature of water, high diversity of species phytoplankton and the prevalence shown high number of individuals in each species, it means water quality in nature of pond is in range suitable with growth of cultivation organisms [2]. Excess density of plankton shown discrepancy in aquatic condition, increase of plankton density at daylight can cause high saturation level of oxygen to be 250% and generate Eboli gas in gill leave system of shrimp and evoke death of shrimp. While at night, there will be a lack of oxygen because of high respiration process by phytoplankton [3].

Problem formulated in this research is: Is the influence of ratio dynamic N:P effect on the abundance of phytoplankton in intensive system shrimp pond. The aim of this research is to discover the influence of ratio dynamic N:P towards abundance of phytoplankton in intensive system shrimp pond.

The benefit of this research is to provide information related to influence of ratio N:P conversion in affecting diversity and abundance of phytoplankton in one of intensive system shrimp ponds, therefore the potential and thread from various species of phytoplankton can be predicted.

## 2. Methodology

### *2.1 Tools and Materials*

Tools being used in this research are: DO meter, spectrophotometer, secchi disk, refractometer, pH pen, plankton net, bucket, ropes, haemocytometer, drop pipette, microscope, glass object, glass cover, sample bottle, burette, stative, clamp, measurement glass 100 ml, Erlenmeyer glass 300 ml, Erlenmeyer glass 500 ml, volume pipette 2 ml dan filler/bulb. The materials being used in this research are: water sample of intensive vannamei shrimp pond PT. Surya Windu Kartika in Banyuwangi Regency, PP indicator, 1%, aquadest, ammonia test kit, reagent of water hardness, nitrite, nitrate and phosphate.

### *2.2 Research Methods*

Research methodology used is surveyed, which is a research method aimed to describe exist and ongoing phenomena. The research was applied without manipulate or change free variables, but describe real condition [4]. Research plan was done by measurement of water quality in 3 sample points include to observe water quality and 12 sample points to observe an abundance of phytoplankton. A sample is taken in 12 sample points in intensive shrimp pond PT. Surya Windu Kartika. Samples to observe water quality of DO, temperature, pH, salinity, water level, abundance and diversity of plankton, and were taken twice a day at 4 PM depends on the availability of sunlight. The measurement of water quality parameters of ammonium, ammonia, nitrite, nitrate and phosphate applied once a day at 7 AM. Samples plankton were taken with plankton net, and then been observed under a microscope with a direct measurement method using Sedgwick Rafter at 100 to 400 times of enlargement, and twice repeated. Water quality observation of ammonium, ammonia, nitrite, nitrate and phosphate is directly performed in 3 sample points using sample bottle, and then observed using spectrophotometer that adjusted to the water. Used in this research is surveyed. The research, preparation was applied to preparing necessary tools and materials, i.e. pH paper, secchi disk, plankton net, bailer, hand counter, haemocytometer, droping pipette, microscope, object glass, cover glass, test kit, sample bottle and lugol. Water samples as material to observed diatom abundance were taken at 3 stations, which was pond plots with 4 points in each corner of intensive system Vannamei shrimp (Litopenaeus vannamei) pond as data clarification. Samples were taken with filtered water using plankton net, then calculate the density. Samples have to be directly brought to the laboratory to be observed and analyzed. Samples were being observed in 100 and 400 times of enlargement under a binocular microscope with direct calculation using haemocytometer. Samples need to be directly observed to maintain quality of phytoplankton. Samples to observe ammonium, nitrite, nitrate and

phosphate were taken in the morning at 5 AM, while water samples to observe plankton and water quality were taken in the afternoon at 4 PM.

## 3. Results

Result of this research consists of main data and supporting data. The main data are number of ratio N:P and abundance of phytoplankton. And supporting data is water quality; include acidity level (pH) and clarity.

### *3. 1 Grade of N: P Ratio*

Data of ratio N: P was obtained from data of total nitrogen in ammonia, ammonium, nitrate, and nitrite, the compared with phosphate, then it became grade of ratio dynamic N: P. Data used is the result of every 3 days calibration. Whereas the result of ratio dynamic N: P can be seen in following graphic:

The measurement result of N: P ratio movement in plot 1 shown stable level of nitrate at DOC 33-48, and high fluctuated at DOC 15 by 15 mg/L and then decrease again in the next day. Dynamic condition ammonium occurred in plot 1 with adequate fluctuate and often frequent contact of ammonium and nitrate at DOC 27-30, 30-34, 45-48 and 55-58. Besides that, in plot 1 was occurred high level of ammonium and nitrate at DOC 31, 49, 52, and 55. The phosphate level in plot 1 was generally stable and increased at DOC 46-61.

The measurement result of nitrite and phosphate in plot 4 shown high dynamic situation at DOC 51 until end of observation session, nitrite increase occurred in DOC 51, 57 and 60 with highest level of nitrate by 25 mg/L at DOC 60. Level of nitrite as intermediate nutrient was significantly increased at DOC 60 of 20 mg/L, other nutrients in plot 4 didn't occur significantly dynamic condition. Nitrite level in plot 4 significantly increased to be 20 mg/L, above threshold of 1.0 mg/L, this can be too dangerous to living organisms in this aquatic system [5].

Observation result of the dynamic situation of nitrate and phosphate in plot 7 shown there was no significant dynamic situation from initial observation until DOC 48. Increased and decreased levels of ammonia, nitrite, nitrate and phosphate at DOC 24-48 to be about 0.1 – 3.5 mg/L. Dynamic level of nitrate significantly increased at DOC 57 and 60 to be the highest one of 60 ml/L, while other nutrient such as ammonia, nitrite, nitrate and phosphate didn't significantly increase only about 5-15 mg/L at DOC 51-60.

### *3.2 Plankton Abundance*

Discovered phytoplankton in all observation stations consists of 19 genus of 4 classes. Those 4 classes are Cyanophyceae (6 genus), Bacillariophyceae (9 genus), Chlorophyceae (3 genus), and Dinophyceae (1 genus). All discovered genus could be seen at table 4:

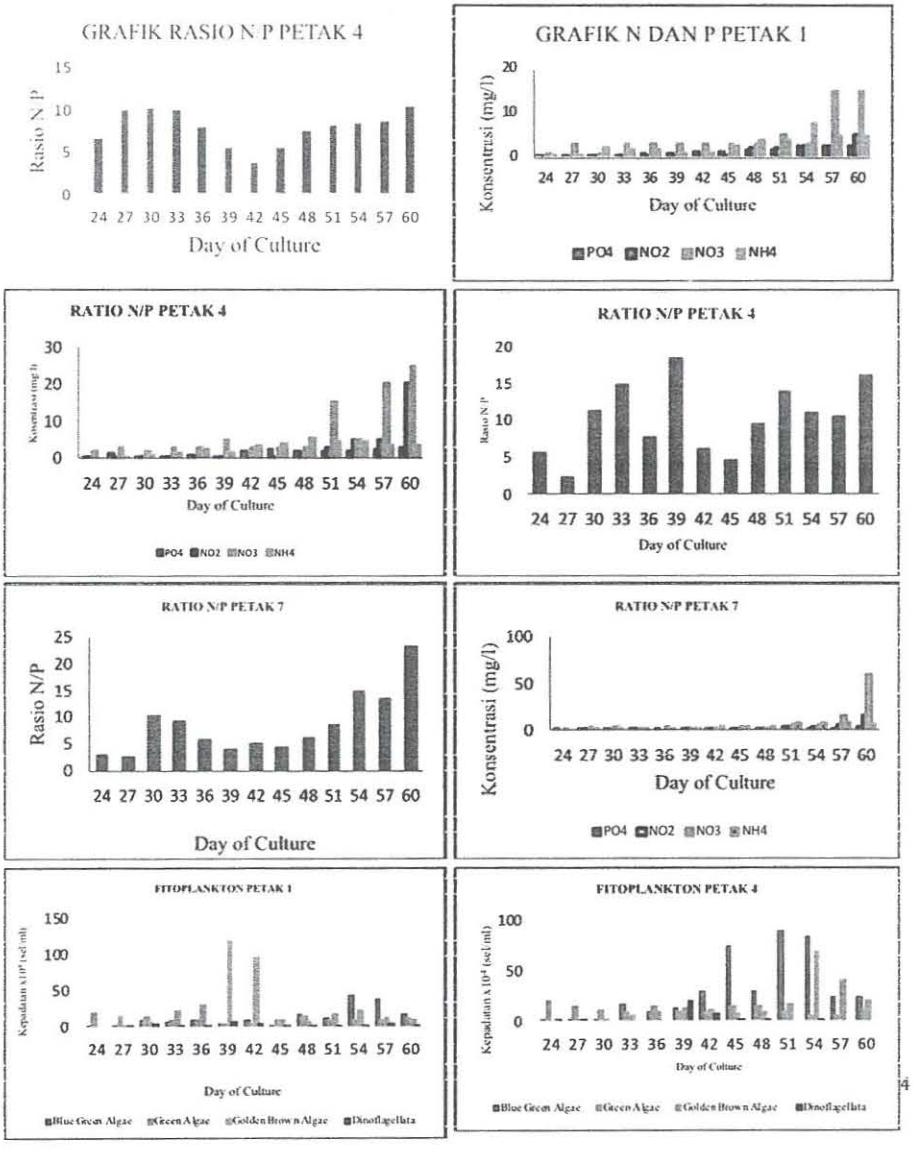
Abundance of phytoplankton in this research consists of density level in total number of cells in the water and percentage composition of phytoplankton in the water. The data are taken from observation in every 3 days. The result of observation can be seen in these following graphics:

Observation result of phytoplankton abundance in plot 1 was generally dominated by phytoplankton of Bacillariophyceae class. The increased of the dynamic situation of Bacillariophyceae started at DOC 30 and gradually increased until achieved highest density at DOC 39, then decreased with not significant dynamic situation until the end of observation session. Significantly increased phytoplankton was also occurring in a class of Cyanophyceae, which increased significantly at DOC 54. Phytoplankton of Clorophyceae and dinoflagellate didn't have significant condition, but the existence in plot 1 always consistent. This shows the stability condition of water in plot 1. Which have a significant dynamic situation at an end observation session at DOC 54 to almost finish.

Observation results of phytoplankton abundance in plot 4 shown high dynamic conditions between phytoplankton of class Cyanophyceae. Phytoplankton of class Cyanophyceae started to have the dynamic condition at DOC 42 and achieved highest density at DOC 51, it is shown dynamic condition of Cyanophyceae is matter in decreased aquatic condition. In plot 4 the dynamic condition of

phytoplankton of class Bacillariophyceae was generally stable, with the highest density at day 54. In general highest abundance of phytoplankton in plot 4 occurred at DOC 54, with domination of class Cyanophyceae and Bacillariophyceae.

Observation results of phytoplankton abundance in plot 7 shown highest density are phytoplankton of class Bacillariophyceae at DOC 39. Beside that phytoplankton of class Bacillariophyceae occurred significantly fluctuate condition from DOC 33 to 39, with fluctuate increased and decreased until end of observation session. Phytoplankton class of Blue green algae didn't occur dynamic condition and more stable compared to Bacillariophyceae, highest growth of Cyanophyceae occurred at DOC. In plot 7, phytoplankton of class dinoflagellate highly increased at DOC 54.



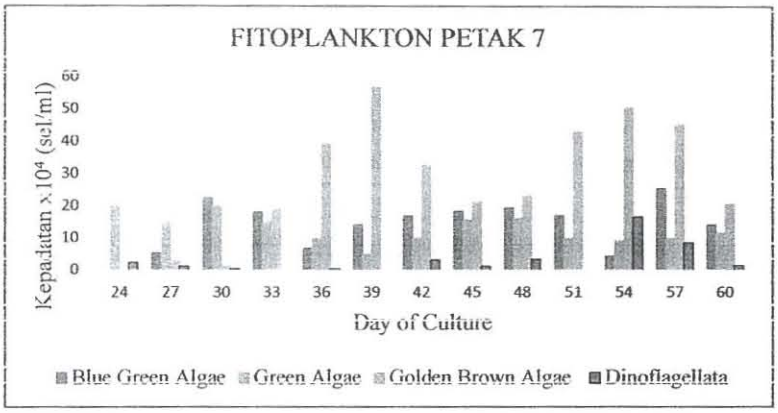



Figure 1. (1) N: P ratio of plot 1 during research, (2) Ammonium, Nitrite, Nitrate and Phosphate Plots 1 during the study, (3) N: P ratio of plot 4 during research, (4) graphs of Ammonium, Nitrite, Nitrate and Phosphate Plot 4 During Research, (5) N: P Ratio 7 during Research, (6) Ammonium, Nitrite, Nitrate and Phosphate Plot 7 During Research, (7) Phytoplankton Plot 1 During Research, (8) Phytoplankton Plot 4 During Research, (9) Phytoplankton Plot 7 During Research

**4. Discussion**

Diatom Nutrients in the aquatic system are important in the existing life of microorganisms, in particular in aquatic cultivation where the existence of microorganisms like phytoplankton and bacteria is important in the success cultivation activities. The most influencing nutrients in the live of phytoplankton are divided into macro elements and microelements, which is determined based on need of phytoplankton towards the elements. Among various nutrients there are 2 most important nutrient to play role as a divider factor in the live of phytoplankton i.e. N and P [6].

N and P are important nutrients in the viability of phytoplankton, both in water in general and in cultivation water. the two nutrients play role as media energy transfer in the cells and also as an essential element in formulating of phytoplankton cells [7]. The influence of N and P is not only limited in ratio dynamic N:P in the water, but also a dynamic form of each element, like ammonium and nitrate in water and dynamic condition of phosphate and nitrite.

Nitrate and ammonia are most dominant form of N elements in influencing the dynamic condition of phytoplankton in the water. It's because nitrate is formed of N to be directly absorbed by phytoplankton, while ammonia is initial material in the nitrification process in cultivation water to stimulate growth of certain phytoplankton. According to previous studies [6] increase content of nitrate in aquatic system able to increase domination of Bacillariophyceae and decrease domination of phytoplankton class of Cyanophyceae, meanwhile increase of ammonia in water is potentially decreasing population of phytoplankton class of Bacillariophyceae and increase density of phytoplankton class of Cyanophyceae.

Based on the research, observation data in 3 observation station, i.e. plot 1 DOC 33 and 51; plot 4 DOC 27, 33, 51 and 57; and plot 7 DOC 27, 36 and 51 shown enhancement of nitrate level in cultivation water affected on increase domination of phytoplankton class of Bacillariophyceae and decrease phytoplankton class of Cyanophyceae. Besides that, decrease of nitrate level affected on the decrease of Bacillariophyceae domination in the water. It is suitable to statement of previous study [6] of the enhancement level of $NO_3$ able to increase abundance of Bacillariophyceae in an aquatic

system.

Based on result of data analysis of the correlation of decrease dynamic condition and dynamic abundance of phytoplankton, there are 4 conditions that lead to phytoplankton dynamic change in the water, they are crashing between $NO_3$ and $NH_4$ in the water that caused dynamic change of Bacillariophyceae like in plot 1 at DOC 30 and 54; pot 4 at DOC 30; and plot 7 at DOC 33 and 39. Beside that the enhancement of $PO_4$ is influencing the dynamic condition between nitrate level and phytoplankton like in plot 1 DOC 54 and in plot 7 DOC 39. It's because the enhancement of $PO_4$ levels in water affected on the decrease of ration N:P as supporting to Bacillariophyceae growth . Significant enhancement of $NO_2$ levels is important to influence dynamic nitrate toward the dynamic condition of phytoplankton in water, like in plot 4 DOC 54 and 16; and plot 7 DOC 57 and 60. It happened because $NO_3$ in level of 1.0 ppm is toxic toward aquatic organism, including phytoplankton [5].

Ammonia is an organic compound that influences the dynamic condition of phytoplankton in water, in particular cultivation water, because the existence of ammonia in the water in general is increasing along the increase of age of the cultivation. According to previous studies [6] enhancement of ammonia level in the water able to decrease phytoplankton class of Bacillariophyceae and increase domination of the class of Cyanophyceae, it is suitable for the observation result in plot 1 at DOC 30, 42, 48 and 54; plot 4 at DOC 30, 33, 42 and 45; and plot 1 DOC 39, 42, 48 and 51. Where ammonia level increased and followed by domination enhancement of Blue green algae. Decreased level of ammonia in the water is also shown decrease domination of phytoplankton class of Cyanophyceae. It is suitable for observation data in plot 1 at DOC 33, 39 and 57; plot 4 at DOC 57; and plot 7 at DOC 36 and 60.

There are several conditions that lead to an exception of correlation ammonium and phytoplankton dynamic condition such as an intensive fluctuated condition of nitrate level in the water so that the influence domination of Cyanophyceae in the water. It possible happens because high levels of nitrate may press Cynophyceae growth, like in plot 4 DOC 27 and 60. Meanwhile, in conditions of less nitrate level or crash nutrient occurred between ammonium and nitrate can push Cyanophyceae growth. $PO_4$ dynamic condition in the water is also important in phytoplankton domination in the water like in plot 4 DOC 39 and 48. Cyanophyceae domination is more affected by availability of $PO_4$ than ammonia in that aquatic system. This is suitable to previous study [8] that water condition with $PO_4$ levels higher than 1.10 ppm can cause a phytoplankton domination class of Cyanophyceae.

A condition where water nutrient is not consumed by phytoplankton can be seen in plot 1 DOC 39, 57 and 60; plot 4 DOC 39, 57 and 60; and plot 7 DOC 60. In this condition nitrate as dividing factor is abundant, but phytoplankton in general decreased or increased of phytoplankton class of dinoflagellate as bio-indicator of discrepancy in aquatic systems. Therefore, nutrient concentration in water is not fully absorbed by phytoplankton. Generally, nutrient cannot be absorbed because of several factors, such as: low level of DO, high pH in the water, high level of nitrite, high level of ammonia, and also significant change of water temperature [8].

## 4. Conclusion

Based on the result of data analysis and discussion related to the correlation of ratio dynamic N:P towards the abundance of phytoplankton, it can be concluded that: value of ratio N:P influence composition of phytoplankton class in the water of Vannamei shrimp cultivation. Dynamic composition of ammonia, nitrite, nitrate and phosphate is influencing dynamic composition of phytoplankton in aquatic systems. Enhancement of nitrate level is affecting increasing of Bacillariophyceae and decrease abundant composition of Cyanophyceae. Enhancement level of ammonia in the water can increase abundant composition of Cyanophyceae and decrease abundant composition of Bacillariophyceae. And the dynamic condition of $PO_4$ is influencing dynamic composition of phytoplankton abundance class of Bacillariophyceae and Cyanophyceae.

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# Dynamic Ratio Correlation N:P toward Diatom Abundance in The Intensive System Vannamei (*Litopenaeus vannamei*) Shrimp Pond

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**Abstract**. Phytoplankton is very expected to grow optimally in water pond. The general phytoplankton management held with optimizes the organic compounds. Diatom is one type of aquatic phytoplankton that has important role. Diatom or *Bacillariophyceae* is phytoplankton which suits to need of shrimp cultivation in the pond because diatom is abundant primary producer and needed as natural feed. Diatom is also potential as better bio-indicator compared to other types of organisms. Diatom needs nitrogen and phosphate in its life, while the nutrient itself in the water doesn't always in always in stabile condition. The research aimed to seek dynamic ratio correlation N:P toward diatom abundance in intensive system vannamei (*Litopenaeus vannamei*) shrimp pond to maintain aquatic stability or living media in this intensive system vannamei shrimp cultivation. The main parameters to be observed are Ammonium, Nitric, Nitrate, Phosphate, as well as abundance of diatom plankton and the supporting parameters to be measured are pH and clarity in the intensive vannamei shrimp pond in Banyuwangi. Based on result of data analysis and study about correlation of dynamic ration N:P toward abundance of Diatom, it can be concluded that ratio value N:P influence the composition of level classification of phytoplankton in aquatic cultivation. The differences level of ammonium, nitric, nitrate and phospate provide different influence into diatom abundance in the water. in the situation of high level of nitrate indicate abundance of diatom.

## 1. Introduction

At the present time Vannamei shrimp (*Litopenaeus vannamei*) is one of important fishery products. Production of Giant tiger prawn agroindustry in Indonesia is decreasing, thus development of Vannamei shrimp is a relevant cultivation alternative [4]. Water quality management is important to success shrimp cultivation. Water quality is determining the availability of various organisms in pond ecosystem, both for cultivating organisms and other biota as compiler of the pond ecosystem. Phytoplankton is one factor in influencing water quality. Phytoplankton production in intensive cultivation is influenced by availability of nutrient in the aquatic system, mostly nutrient from group of Nitrogen (N) and Phosphate (P) [2].

Phytoplankton is expected to grow optimally in a water pond. In general phytoplankton management is performed by optimize organic compounds, use fertilizer and water change [2]. Nitrogen and phosphor are two-influence parameters in the water. There are many nutrients in the water, but only some of them able to be utilized by phytoplankton [3].

Diatom is one important species of phytoplankton in the water. Diatom of Bacillariophyceae is a phytoplankton that is suitable to need of shrimp cultivation at the pond [17]. Diatom is also potential as a better bio-indicator compared to other organisms [15]. Growth supporting factor of plankton is complex and interact each other between physic and chemistry factors in the water like dissolved oxygen, temperature and availability of nitrogen and phosphor [13].

Existence of diatom in an aquatic system is influenced by various aspects, one of them is the availability of ratio N:P, while these nutrients in the water are not stable. Regarding to the condition above then it is necessary to apply further research of correlation of ratio dynamic N:P the towards abundance of diatom in Intensive system Vannamei shrimp (*Litopenaeus vannamei*) pond to maintain water stability or living media in intensive system Vannamei shrimp pond.

Problem formulating in this research is: how is the correlation of ratio dynamics N:P towards diatom abundance in intensive system Vannamei shrimp *(Litopenaeus vannamei)* pond. The aim of this research is to discover the correlation of ratio dynamic dynamics N:P towards diatom abundance in intensive system Vannamei shrimp (*Litopenaeus vannamei*) pond in order to maintain water stability or living media in intensive system Vanamei shrimp cultivation.

The benefit of this research is to provide information for the readers about the correlation of dynamics N:P towards diatom abundance in intensive system Vannamei shrimp (*Litopenaeus vannamei*) pond. Beside that, the information of this research result is useful to develop knowledge and its application for the community, in particular Vannamei shrimp farmers.

## 2. Methodology

### *2.1 Tools and Materials*

The tools being used in this research are pH paper, secchi disk, plankton net, bailer, hand counter, haemocytometer, drop pipette, microscope, object glass, cover glass, test kit, and sample bottle. The materials being used in the research are sample water of intensive system vaname shrimp (*Litopenaeus vannamei*) pond and lugol to deactivated plankton movement.

### *2.2 Research Methods*

The method used in this research is surveyed. The research preparation was applied with prepare necessary tools and materials, i.e. pH paper, secchi disk, plankton net, bailer, hand counter, haemocytometer, drop pipette, microscope, object glass, cover glass, test kit, sample bottle and lugol. Water samples as material to observed diatom abundance were taken at 3 stations, which was pond plots with 4 points in each corner of intensive system Vannamei shrimp (*Litopenaeus vannamei*) pond as data clarification. Samples were taken with filtered water using plankton net, then calculate the density.

Samples have to be directly brought to the laboratory to be observed and analyzed. Samples were being observed in 100 and 400 times of enlargement under a binocular microscope with direct calculation using haemocytometer. Samples need to be directly observed to maintain quality of phytoplankton. Samples to observe ammonium, nitrite, nitrate and phosphate were taken in the morning at 5 AM, while water samples to observe plankton and water quality were taken in the afternoon at 4 PM.

*2.3 Calculation and Observation of Diatom*

Diatom observation consists of identify and abundant in the pond. Diatom observation is applied in 3 plots of the pond, and taken at 4 points in the corners for each plot. Samples of diatom observation were taken by some water in 4 points in the corner using plankton net with a mesh size of 20 microns. Plankton net with a mesh size of 20 microns is able to filter phytoplankton class of diatom, as well as enable water to come out through the micro hole of the plankton net.

The method used in the identification and observation of phytoplankton was a direct calculation using haemocytometer. It was applied by taking 1 ml water sample from the bottle sample, then cover it with glass cover. Observation was done by identifying the phytoplankton and calculate its density in the haemocytometer. Sum of phytoplankton was calculated using the method that suitable for the size of the plankton. Samples have to be directly calculated to maintain quality of observed phytoplankton, to make easier to identify and density calculation when observing under microscope.

## 3. Results and Discussion

The result of this research consists of ratio dynamic N:P and plankton abundance as main data, and parameter of water quality like level of acidity (pH) and water clarity as supporting data. Observation was applied in 3 plots of intensive system Vanamei shrimp pond.

*3.1 Grade of N:P Ratio*

Data of ratio N:P is obtained from data of total nitrogen in ammonium, nitrite and nitrate and compared to phosphate to getting dynamic ratio N:P in 3 stations/plots. Data is taken from observation every 3 days. The result of calibrate dynamic ratio N:P can be seen in the following graphics bellows:

Based of the graphic above, ratio N:P in plot 4 at day 24$^{th}$ to day 27$^{th}$ was decreased, then it increased at day 27$^{th}$ to day 33$^{rd}$. on the next day at day 33$^{rd}$ to day 36 the ratio N:P decreased then started to increase again at day 39$^{th}$ and after it the ratio N:P decreased until day 45$^{th}$. And at day 45$^{th}$ to day 54$^{th}$ the ratio N:P increased. At day 51$^{st}$ to day 57$^{th}$ it decreased, then increased before day 60$^{th}$. The cause of fluctuate grade of N:P ratio is change level of ammonium, nitrate, nitrite and phosphate as mentioned below:

Calculation of ration N:P in plot 4 resulted various fluctuate situation, at shrimp age of day 24$^{th}$ to 30$^{th}$ it increased, then decreased day 30$^{th}$ to day 39$^{th}$. Then at shrimp age of day 30$^{th}$ to 42$^{nd}$ the ratio N:P increased then slightly decreased at day 45$^{th}$. At day 45$^{th}$ to day 54$^{th}$ the ratio N:P increased, then at day 54$^{th}$ to day 57$^{th}$ it decreased, and then increased again until day 60$^{th}$. The cause of fluctuate grade of N:P ratio is change level of ammonium, nitrate, nitrite and phosphate as mentioned below:

*3.2 Plankton Abundance*

Plankton with high abundance is species who able to support its live more efficient than other species in same tropical level. In means that species have important role for the plankton community in that aquatic system(Qiptiyah et al., 2008). The observation was applying in 3 different stations/plots. Observation data was result of every-3-day calibration. It can be shown in the following graph bellow:

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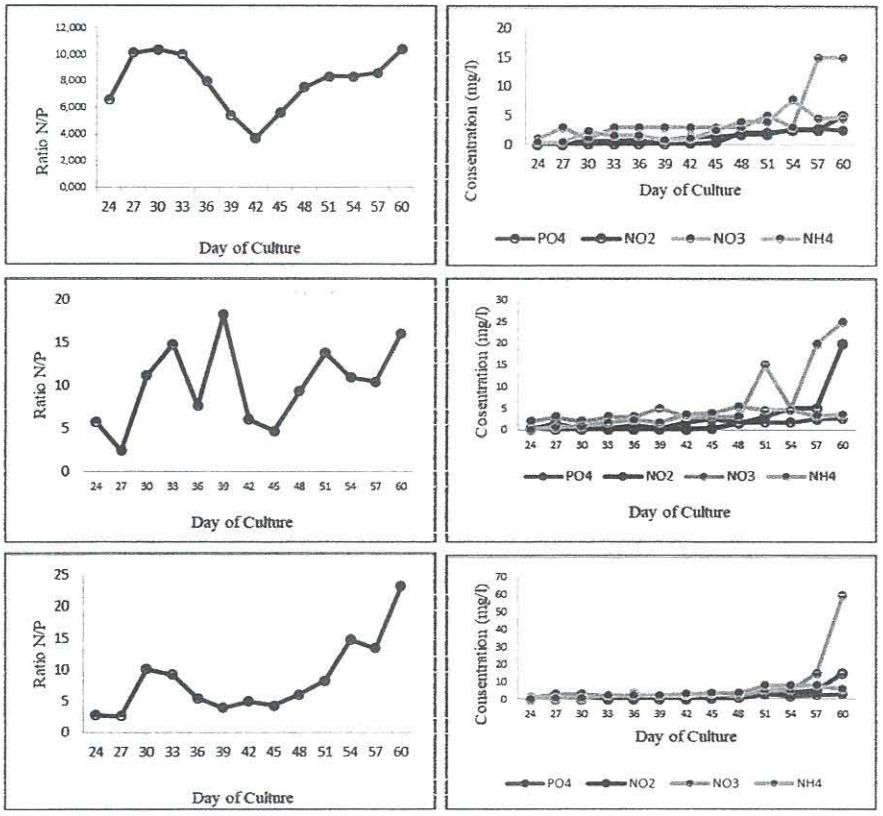
Based on the graphic of the diatom journey above, it's shown that in initial day the density of diatom remained low in level of 0 cells/mL at day 24$^{th}$. And at day 24$^{th}$ to day 39$^{th}$ the diatom density has

continually increased. Highest diatom density occurred at day 39th to be 1,187,500 cells/mL. Diatom density was decreasing at day 39th to day 48th, then increased again until day 54th. At day 57th to day 60th the diatom density decreased again.

Based on the graphic of diatom density in plot 4 shown in initial at day 24th the diatom density remain to be 0 cells/mL which shown less density at the initial cultivation. Then at day 24th to day 39th the density increased to be 135,000 cells/mL. At day 39th to day 45th it decreased, and then at day 45th to day 54th diatom density was continually increasing. The highest level of diatom density in plot 4 was at day 54th to be 682,500 cells/ml. Then the density decreased until day 60th.



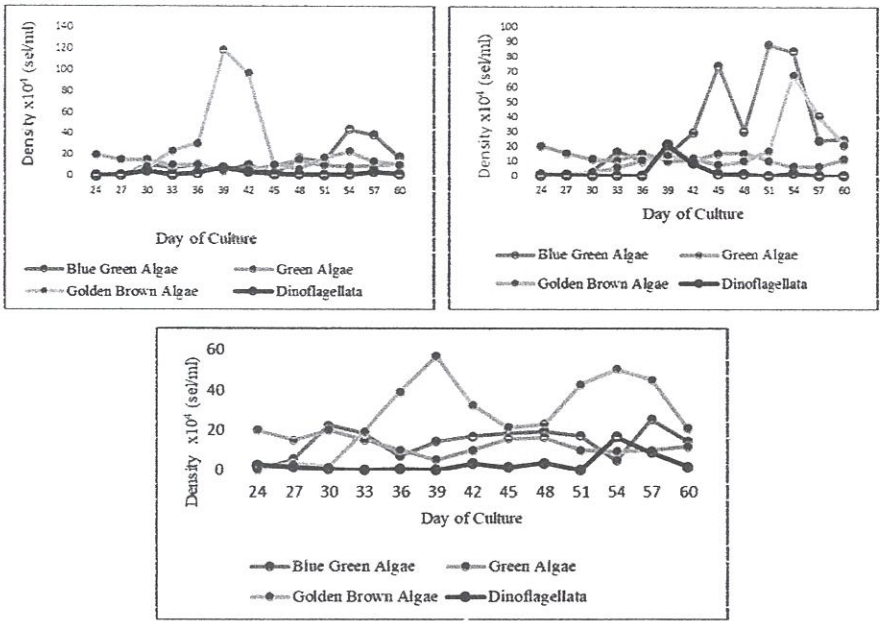



**Figure** 1) Ratio N: P at Plot 1 During Research, 2) Plot 1 Ammonium, Nitrite, Nitrate and Phosphate During Research, 3) Ratio N: P Plot 4 During Research, 4) Plot Ammonium, Nitrite, Nitrate and Phosphate 4 During Research, 5) Ratio N: P Plot 7 During Research, 6) Ammonium, Nitrite, Nitrate and Phosphate during Research, 7) Phytoplankton Chart Plots 1 During Research,8) Phytoplankton Chart 4 during Research, 9) Phytoplankton Chart 7 during Research

Based on the graphic above, the density of diatom was increasing at day 24th to day 27th, and then decreased until day 30th. Density of diatom increased again at day 30th to day 39th, and then decreased until day 45th. Lowest level of diatom density in plot 7 was at day 24 of 2,500 cells/mL and highest level occurred at day 39th to be 570,000 cells/mL. At day 45th to day 54th diatom density increased, and then started to decrease until day 60th.

Diatom or Bacillariophyceae is suitable phytoplankton to the need of shrimp cultivation at the pond [17] because diatom is abundant primary producer and needed as natural feed in both fresh water and the sea [1]. Diatom is suitable for larva, shrimp, fish and mussel, because it contains complete unsaturated fatty acid, vitamins and amino acid better than synthetic feed [16]. Diatom is also able to be a bio-indicator for water contamination because it has wall cells from silica. Wall cells from silica in general is strong and remain intact, thus analysis of wall cells will indicate accumulated contamination particles in aquatic system (Amedia, 2013 in Kamilah et al., 2014).

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Nutrients are necessary parameter to the living and growing of microalgae, they are in the form of chemical compound needed in metabolism process and cannot be produced by the organisms but obtained from the nature. Organisms need nutrient to develop and improve body tissue, manage processes inside the body as well as provide energy for the body. The availability of nutrient is basically the availability of N and P. Nutrients source of N and P is fertilizer and waste. Usage of N basis fertilizer (like Urea, $(NH_2)_2CO$) in area of globally cultivation has been increased by 100 times of N source in the water [8]. Nitrogen for plant or algae is mostly used in chlorophyll, which is important for photosynthesis and further growth [7]. The phosphor is very important for viability water organisms because its function in storage and transfer energy in the cells and useful in the genetic system [11].

Ratio N:P is one important factor in the pond and one of the success determinant in activities of cultivation, in particular shrimp cultivation. It is in line with [6] that high concentration of nutrients in the water will affect in aquatic system productivity, composition of nutrients of ratio N:P is well-known as a red-field ratio. N:P ratio is strongly affecting plankton abundance. Ratio N:P can be calculated with data combination of ammonium, nitrite and nitrate as component N and phosphate of P.

Ammonia resulted from shrimp feces, leftover feed and died phytoplankton. Ammonia in water is in two forms of not ionized (ammonia, $NH_3$) and ionized (ammonium, $NH_4$). Ionized ammonia is harmful nutrient for water organisms because of the toxic, while not ionized ammonia can be used as a nitrogen source by phytoplankton [5].

Nitrite in the water often found in less amount because it's unstable [6]. Nitrite concentration is less detected in the water, it because of the nitrification process by nitrosomonas bacteria [6]. According to natural water contains nitrite of 0.001 mg/L and it should not more than 0.006 mg/L. Above 0.05 mg/L nitrite is toxic for sensitive organisms in water [5]. This is in line with the condition in plot 1, plot 4 and plot 7 at last observation day or day 60$^{th}$, where extremely high nitrate concentration caused diatom density deprivation. Nitrate is a major form of nitrogen in natural water and main nutrient for plant or algae growth. It is dissolved easily and unstable [5]. [12] mentioned that for optimal growth phytoplankton need nitrate in the range of 0.9 – 3.5 mg/L.

An aquatic system with low phosphate concentration (0.00 – 0.02 mg/L), will be dominated by phytoplankton class of Bacillariophyceae (Diatom). In medium phosphate concentration (0.02 – 0.005 mg/L), will be dominated by class of Clorophyceae. And in high phosphate concentration (>0.010 mg/L), will be dominated by a class of Cyanophyceae. Orthophosphate is a form of phosphate that able to be directly utilized by aquatic plan for growth of phytoplankton. Phosphate usually available in fewer amounts thus can be divider factor for growth of phytoplankton [14].

Based on the observation result of phytoplankton during the research, there were various species of phytoplankton of class Blue Green Algae, Green Algae, Golden Brown Algae and Dinoflagellata. This is in line with [20] statement of ratio N:P in the water will influence the dominant composition of plankton species. Ration N:P of >20 mean the nature will be dominated by plankton diatom (Golden Brown Algae), while the ratio N:P of approximate 20 mean the nature will be dominated by Green Algae, and ratio N:P <10 means nature is suitable for Blue Green Algae.

Based on the observation result along 60 days in 3 plots, it is obtained that different level of nitrogen and phosphor give different influence for phytoplankton composition. Abundance of diatom in initial cultivation at day 24$^{th}$ was remaining few in three plots by 0 – 2500 cells/mL, it possibly happen because of low level of nutrient in the pond. It is suitable with a statement of [18] that along with the increase of cultivation time, then accumulated of leftover feed and shrimp feces will also increase, both of them are organic compounds which then will be decomposed by bacteria to be inorganic compounds (such as ammonia, nitrate, nitrite and orthophosphate) so that the water will be more fertile. Fertile enhancement as a result of accumulated left over feed and shrimp feces will affects to increase of diatom abundance into highest level of 1,187,500 cells/L.

Observation in plot 1 at day 27$^{th}$, 30$^{th}$, 42$^{nd}$ and 51$^{st}$; in plot 4 at day 30$^{th}$, 33$^{rd}$, 39$^{th}$, 42$^{nd}$, 45$^{th}$, 48$^{th}$, 51$^{st}$ and 57$^{th}$; and in plot 7 at day 45$^{th}$, 48$^{th}$, 51$^{st}$, 54$^{th}$ and 57$^{th}$. It discovered that increase and decrease of ratio N:P is suitable to the increase and decrease of diatom density level.

Ratio N:P in plot 1 at day 33$^{rd}$, 36$^{th}$, 45$^{th}$, 48$^{th}$, 54$^{th}$, 57$^{th}$ and 60$^{th}$; in plot 4 at day 27$^{th}$, 36$^{th}$, 54$^{th}$ and 60$^{th}$; and in plot 7 at day 27$^{th}$, 30$^{th}$, 33$^{rd}$, 39$^{th}$, 42$^{nd}$ and 60$^{th}$. It is discovered increase and decrease of ratio N:P is not in line with increase and decrease of diatom density level, it can be seen in graphic ratio N:P and graphic of phytoplankton density during the research in plot 1, 4 and 7. This has possibly happened because the different composition level of ammonium, nitrite, nitrate and phosphate. According to [12] that abundance of diatom can be influenced by nutrients like Nitrate and Phosphate.

Nitrate fluctuates condition is influencing increase of diatom density, it can be seen in plot 1 at day 27th and 33rd; plot 4 at day 27th, 33rd and 51st; and in plot 7 at day 24th, 36th and 51st where increase nitrate was causing increasing diatom density. It is suitable with statement of [12] that nitrate ($NO_3^-$) is the main nutrient for diatom to grow and develop well. The high concentration of nitrate in the water will stimulate growth of diatom, because nitrate in certain concentration gives good condition for diatom to grow.

In several conditions with low level of nitrate, but the level of diatoms is increasing, like what happen in plot 1 at day 30th, 51st and 54th; plot 7 at day 33rd and 39th. It can be possibly occurred because in that condition other class of phytoplankton such as Blue Green Algae and Green Algae also need nutrients. However at this condition the phytoplankton decrease, thus they can't absorb nutrient very well. It means the nutrients were more utilized by diatom.

As usual phosphate is available in a few level so it can be used as dividing factor for phytoplankton growth [14]. In plot 1 at day 51st to day 60th; in plot 4 at day 57th of days 60th was having an increase in phosphate level, this caused an abundance of phytoplankton is dominated by Blue Green Algae rather than diatom. This is suitable with a statement of [12] that aquatic system with low concentration of phosphate will be dominated by phytoplankton of class Bacillariophyceae (Diatom) and in high concentration of phosphate (>0.10 mg/L) will be dominated by Blue Green Algae.

Based on plot 1 data, plot 4 and plot of 7 phytoplankton diatom density were more prevalent based on [9] describe Bacillariophyceae as a type of diatom that is more tolerant of environmental conditions such as temperature and able to adapt well to the environment that reproduces quickly and well. When there is an increase in nutrient levels, diatoms are able to do mitotic divisiSon three times in 24 hours. Dinoflagella can only be used once in 24 hours in the same nutrient conditions.

In diatom cultivation activities are also able to adapt well by regulating spores in accordance with the statement stated by [10] that the Bacillariophyceae class also has good adaptability one can function silent spores which are usually smaller than other diatom cells. This silent spore can survive and grow in poor conditions as well as the parameter environment which is still relatively stable for phytoplankton growth from the Bacillariophyceae class.

## 4. Conclusion

These results indicate that the relationship of dynamic data the ratio of N: P to the abundance of diatoms can be concluded which; N: P ratio values affect the composition of phytoplankton classes under cultivation. Differences in the value of ammonium, nitrite, nitrate and phosphate have different effects on diatom abundance on the continent. Under high nitrate conditions, it indicates the value of abundance in diatom phytoplankton or golden brown algae.


## 5. Acknowledgments

We are thankful to our team of researchers, technicians of the yield of ponds for their valuable help with field work and sample collection.


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Commented [A3]: should references be written not in alphabetical order, please refer to the format again


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# Dynamics ratio correlation n: p toward abundance of blue green algae in intensive system of vaname (*Litopenameous vannamei*) shrimp pond

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**Abstract.** Phytoplankton in the intensive system of Vaname shrimp cultivation is useful as indicator on water quality. Phytoplankton influences the growth and productivity of cultivation organism within the pond, thus optimally managing the phytoplankton is necessary. Manage phytoplankton is done by adjusting the levels of organic compounds. Nitrogen and phosphorus are two affect parameters in the water. Blue green algae are group of phytoplankton that have important role, although there are several disserve species. Blue green algae are abundant primary producer and can be found a lot both in freshwater and sea. Blue green algae also potential as bio-indicator to designate how good or bad water condition is. This research aimed to seek correlation of ratio dynamic N:P towards abundance of blue green algae within system of Vaname (*Litopenaeus vannamei*) shrimp pond. The main parameters observed are ammonium, nitric, nitrate, phosphate and abundance of plankton blue green algae, as well as measure the supporting parameters of pH and clarity of the intensive system of Vaname shrimp pond in Banyuwangi. Based on data analysis and study of dynamic ratio correlation N:P toward abundance of blue green algae, it can be concluded that level of ratio N:P influence the level composition of phytoplankton in water cultivation. The difference level of ammonium, nitric, phosphate is influencing the abundance of blue green algae in the water. High-level of ammonium and low level of nitric caused high-level abundance of blue green algae.

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## 1. Introduction

Vaname shrimp can be cultivated using intensive system method. One of characteristic of intensive system cultivation is high density. High density affects on the feed needed, space for movement, and oxygen level, which will influence the quality of caring media, growth and the viability of Vaname shrimp [1].

The excess of feed will increase organic waste in the water. Leftover feed contains of necessary nutrient for plants in order to survive and grow, including phytoplankton. The growth of phytoplankton in the water is influenced by the availability of nitrogen and phosphor. Nitrogen and phosphor are more needed than carbon, hydrogen and oxygen, because nitrogen and phosphor can be utilized by phytoplankton with small quantity [2].

Nitrogen and phosphor level in the nature is limited, so that affects on the growth of phytoplankton. The usage of organic and inorganic fertilizer in cultivation pond is one way to develop water fertile. Fertile water is indicated by the abundance of phytoplankton [3]. The usage of fertilizer with certain ratio N:P can determine the abundance of phytoplankton in an aquatic system. The ratio value N:P of 10:1 or less can bring up the domination of Blue green algae, this possibly happen because Blue green algae can survive in extreme condition, include condition of low level of nitrate. The ideal ratio N:P within the cultivation pond is 16:1 [4].

Blue green algae often experience a population explosion (blooming algae). The phytoplankton explosion of population is caused by excess nutrient in the water. Too many nutrients in the water are known as eutrophication. Eutrophication is caused by natural processes or by contamination due to too many nutrients dissolved in water, or it can be from the waste stream that being discarded to the water or contaminate the cultivation water source. Water with excess nutrients caused phytoplankton growth too fast or uncontrollable, including Blue green algae [5].

Blue green algae also known to able to product toxic, which lead to disrupt productivity of Vaname shrimp. Blue green algae can also be competitor of other plankton existence. Vaname shrimp cultivation pond that contain a lot of harmful Blue green algae will experience productivity disruption, it will lead to the potential death of the cultivation organisms. Blue green algae that often dominates water when population blooming is occurring commonly from harmful genus [5].

Blooming population of Blue green algae is mostly avoided by vaname shrimp cultivators. Therefore this research of "Correlation of Dynamic Ratio N:P Towards Abundance of Blue green algae in The Intensive System Vaname Shrimp (Litopenaeus vannamei) Pond needed to be done to discover the influence of ratio dynamic N:P towards dynamic of Blue green algae in the nature of vaname shrimp pond cultivation.

## 2. Methodology

### *2.1 Tools and Materials*

The tools being used in this research are pH paper, secchi disk, plankton net, bailer, hand counter, haemocytometer, drop pipette, microscope, object glass, cover glass, test kit, and sample bottle. The materials being used in the research are sample water of intensive system vaname shrimp (Litopenaeus vannamei) pond and lugol to deactivated plankton movement.

### *2.2 Research Methods*

The method used in this research is surveyed. The research preparation was applied to preparing necessary tools and materials, i.e. pH paper, secchi disk, plankton net, bailer, hand counter, haemocytometer, drop pipette, microscope, object glass, cover glass, test kit, sample bottle and lugol. Water samples as material to observed diatom abundance were taken at 3 stations. which was pond plots with 4 points in each corner of intensive system Vannamei shrimp (Litipenaeus vannamei) pond as data clarification. Samples were taken with filtered water using plankton net, then calculate the density. Samples have to be directly brought to the laboratory to be observed and analyzed. Samples were being observed in 100 and 400 times of enlargement under a binocular microscope with direct calculation using haemocytometer. Samples need to be directly observed to maintain quality of

phytoplankton. Samples to observe ammonium, nitrite, nitrate and phosphate were taken in the morning at 5 AM, while water samples to observe plankton and water quality were taken in the afternoon at 4 PM.

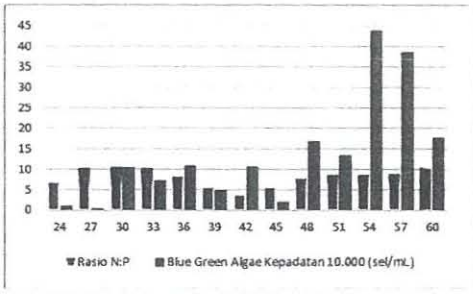
*2.3 Calculation and Observation of Diatom*

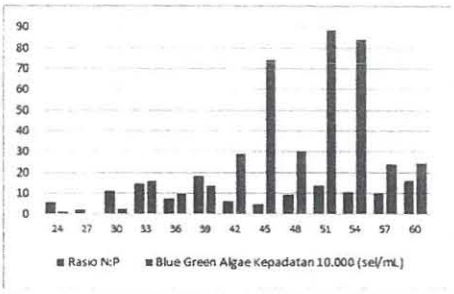
Diatom observation consists of identifying and abundant in the pond. Diatom observation is applied in 3 plots of the pond, and taken at 4 points in the corners for each plot. Samples of diatom observation were taken by some water in 4 points in the corner using plankton net with a mesh size of 20 microns. Plankton net with mesh size of 20 microns is able to filter phytoplankton class of diatom, as well as enables water to come out through the micro hole of the plankton net.

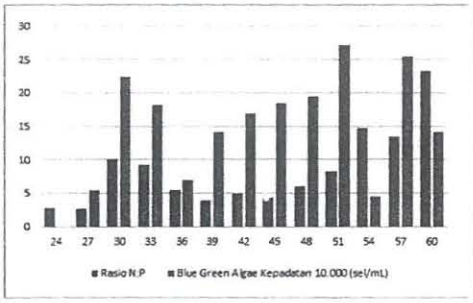
The Method used in the identification and observation of phytoplankton was a direct calculation using haemocytometer. It was applied by taking 1 ml water sample from the sample bottle, then cover it with glass cover. Observation was done by identifying the phytoplankton and calculate its density in the haemocytometer. Sum of phytoplankton was calculated using the method that suitable for size of the plankton. Samples have to be directly calculated to maintain quality of observed phytoplankton, to make easier to identify and density calculation when observing under microscope.

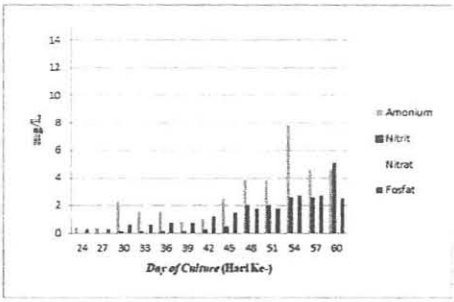
**3. Results and Discussion**

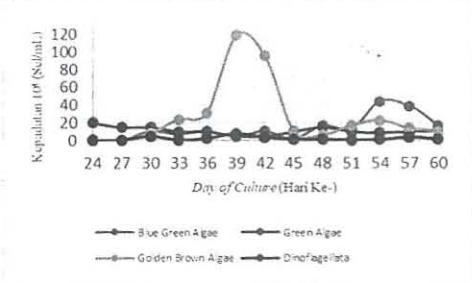
The result of this research Data of ratio N:P is taken from accumulation data of ammonium, nitrite, nitrate to be N and compared to the phosphor to getting the ratio N:P. Result of measurement ratio N:P can be seen in the following figure 1:

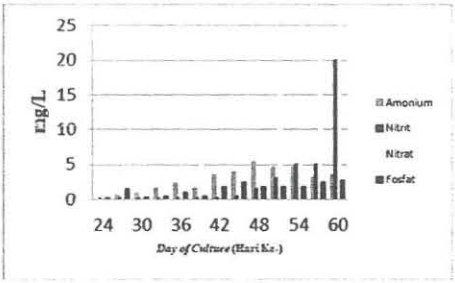

Rasio N:P
Blue Green Algae Kepadatan 10.000 (sel/mL)


Rasio N:P
Blue Green Algae Kepadatan 10.000 (sel/mL)


Rasio N:P
Blue Green Algae Kepadatan 10.000 (sel/mL)


mg/L
Amonium
Nitrit
Nitrat
Fosfat
Day of Culture (Hari Ke-)


Day of Culture (Hari Ke-)
Blue Green Algae
Green Algae
Golden Brown Algae
Dinoflagellata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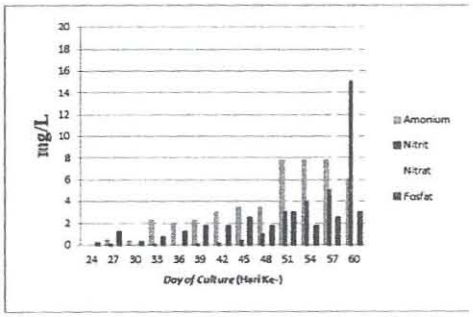
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Day of Culture (Hari Ke-)

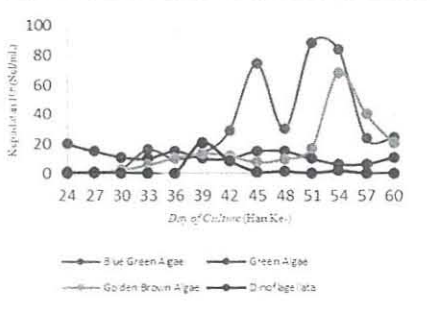
Day of Culture (Hari Ke-)
Blue Green Algae
Green Algae
Golden Brown Algae
Dinoflagellata

**Figure 1**. 1) Comparison of N: P ratio with Blue green algae density in plot 1, 2) Comparison of N: P ratio with Blue green algae density in plot 4, 3) Comparison of N: P ratio with density of green blue algae in plot 7 , 4) dynamics of ammonium, nitrite, nitrate, and phosphate (Plot 1), 5) dynamics of ammonium, nitrite, nitrate, and phosphate (Plot 4), 6) dynamics of ammonium, nitrite, nitrate, and phosphate (Plot 7), 7 ) total phytoplankton dynamics (Plots 1), 8) Charts of total phytoplankton dynamics (Plots 4), 9) total phytoplankton dynamics (Plot 7)

Results of ratio N:P in plot 1 at day 24th of Vaname shrimp cultivation, there was an increase in day 27th, then less increase at day 30th, furthermore ratio N:P was gradually decreasing until the day 42nd. Ration dynamic N:P in the plot 1 increased until the day 60th of the cultivation. Lowest ratio N:P in the plot 1 was in day 42nd, while highest value was at day 60th.

Results of ratio N:P in the plot 4 with more fluctuate values. During the day 24th to at day 27th of cultivation ratio N:P decreased, then increased until day 33rd of cultivation. Ratio N:P decreased at day 36th and back to increase until day 39th. Decrease was occurring again at day 45th, then increased at day 51st. And then ratio N:P decreased at day 57th and increased at day 60th. Dynamic ratio N:P in plot 4 is the most unstable compared to plot 1 and 7. Lowest ratio N:P in plot 4 was in day 27th, while highest ratio was at day 39th.

Results of ratio N:P in plot 7, decreased at day 27th and increased at day 30th. Ratio N:P was decreasing until day 39th, then increased until day 45th. Ratio N:P increased until day 54th then decreased at day 57th, and then increased at day 60th. Lowest ratio N:P in plot 7 was in day 27th, while highest value was at day 60th.

Lowest ammonium level in plot 1 was at day 24th and 27th, while highest ammonium level is at day 54th. Ammonium level was extremely increasing at day 54th, then decrease at day 57. While the nitrite level remained stable at 24th to 45th day, then increased at day 48th, then remained stable until day 57th. Nitrite level was double increasing at day 60th. Lowest nitrite level was at day 24th and 30th, while highest level was at day 57th and 30th. Nitrate was undergone dynamic condition from day 24th to day 33th, then remained stable until day 48th, increased at day 51st and significantly increased at day 57th and 60th. Meanwhile phosphate remained stable. The level of phosphate did not decrease until day 57th, but then decreased at day 60th. Highest level was at day 57th.

Lowest ammonium level in plot 4 was at day 24th, then increased until day 36th, then decreased at day 39th. Level of ammonium was decreasing and achieved the highest level at day 48, then decreased until day 57, then slightly increased at day 60th. Level of nitrite remained stable until day 45th, then increased until day 54. The lower level of nitrite was at day 45th, and the highest level was at day 60th. Meanwhile nitrate was having the dynamic condition at day 24th to day 48th, then significantly increased at day 51st and decreased at day 54th. Nitrate level increased again at day 57th, and achieved the highest level at day 60th. The lowest level of phosphate was at day 24th. Phosphate was having the dynamic condition until day 48th, then remained stable until day 54th. Phosphate level increased until day 60th. Highest level of phosphate was at day 60th.

The lowest ammonium level in plot 7 was at day 24th. Level of ammonium was undergoing dynamic condition until day 45th, then become stable at day 48th. Then in increased until the highest level at day 51st and then become stable until day 57th. The level of ammonium decreased at day 60th. Meanwhile nitrite was achieving the lowest level at day 24th, 30th and 36th. Nitrite level tended to stable until day 45th, then increased until day 60th. Highest level of nitrite was at day 60th and lowest level was at day 24th. Moreover nitrate didn't undergo a substantial dynamic condition until day 57th. Nitrate level rapidly increased and achieved the highest level at day 60th. Meanwhile the lowest level of phosphate was at day 24th. Phosphate level didn't undergo substantial dynamic situation. The highest level of phosphate was at day 51st and 60th.

The lowest density of Blue green algae in plot 1 was at day 27th. Blue green algae decreased from day 24th to day 27th, then increased at day 30th. Blue green algae was decreasing again at day 33rd and back to increase at day 39th. Once more Blue green algae decreased until day 51st and then significantly increased until its highest density at day 54th. Then Blue green algae decreased until day 60th.

The lowest density of Blue green algae in plot 4 was at day 27th. Blue green algae decreased at day 36th and then increased until day 45th. Blue green algae was decreasing again at day 48th, then increased until its highest density at day 51st. Once more Blue green algae decreased until day 57th and then slightly increased at day 60th.

Blue green algae in plot 7 couldn't be discovered at day 24th. Blue green algae started to develop from day 27th to day 33rd, blue green algae decreased at day 36th and increased at day 39th. During the day 39th until day 51st, Blue green algae was increasing. Then Blue green algae decreased at day 54th and increased again at day 57th, and afterward decreased again at day 60th. Highest density of Blue green algae was at day 51st.

## 4. DISCUSSION

Diatom Ratio N:P of intensive system vaname shrimp pond in plot 1 is about 3.7 10.4. This number can be categorized low one in the activities of fish cultivation because the highest number of N:P is 10. This phenomena can be reviewed with statement [6] mentioned that threshold on the contents of ration N:P in the adequate cultivation water is in 16:1. The number of ratio N:P in the plot 4 is about 2.3 – 18.3, which met the standard of cultivation. Meanwhile plot 7 shown ratio N:P in about 2.6 – 23.2, which the upper point surpasses the standard of cultivation but remain normal because ratio N:P of 20:1 will support the growth of diatom. All plots shown dynamic ratio N:P, however plot 4 and 7 are the most ideal condition according to [4], because the most ideal ratio N:P in the aquatic cultivation is 16:1. The level of ratio N:P is influenced by organic compounds. Organic compounds in the water come from microorganisms' body, plants, and the result of organisms' metabolism process [7]. Organic compounds in water are decomposed by bacteria to be N and P, and then can be utilized by organisms. Enhancement of nitrogen level in the water can also be influenced by the derivation of total plankton density [8].

The increase of the ammonium level in the plot 1 occurred at day 30th, 42nd, 45th, 48th and 54th. Meanwhile plot 4 increased at day 27th, 30th, 33rd, 36th, 42nd, 45th, 48th and 60th. Furthermore plot 7 increase occurred at day 27th, 33rd, 39th, 42nd, 45th and 51st. Significant increased in the plot 1 was at day 54th, to be 3.9 mg/L, in plot 4 at day 42nd to be 1.9 mg/L and in plot at day 51st to be 4.3 mg/L. Deprivation of ammonium in plot 1 occurred at day 33th, 39th and 57th, in plot 4 at day 39th, 51st, 57th, And in plot 7 at day 30th, 36th, 60th. Significant deprivation in plot 1 occurred at day 57th to be 3.2 mg/L; plot 4 at day 57th to be 1.4 mg/L; and in plot 7 at day 60th to be 1.8 mg/L.

According to [9], enhancement and deprivation of ammonium are caused by factor of the bacteria's nitrification process. Enhancement ammonium is caused of ammonium can't be oxidized to be nitrite, it possibly happen because Nitrosomonas bacteria are not properly working to recast ammonium. Ammonium deprivation is caused by Nitrosomonas working properly to recast ammonium. Died organisms that cause accumulation of organic compounds is also the cause of high-level ammonium in the water.

Nitrite enhancement in plot 1 occurred at day 30th, 33rd, 42nd, 45th, 48th, 54th and 60th. Enhancement in plot 4 occurred at day 27th, 42nd, 45th, 48th, 51st, 54th and 60th. Enhancement in plot 7 occurred at day 27th, 33rd, 39th, 42nd, 45th, 48th, 51st, 54th, 57th and 60th. Significant deprivation in plot 1 was occurring at day 60th to be 2.5 mg/L; in plot 4 at day 60th to be 15 mg/L; and in plot 7 at day 60th to be 10 mg/L. Nitrite deprivation in plot 1 occurred at day 33rd, 39th, 57th; in plot 4 at day 39th, 51st, 57th; and in plot 7 at day 30th, 36th, 60th. Significant deprivation occurred in plot 4 at day 30th to be 1.4 mg/L; and in plot 7 at day 30th and 36th to be 1.8 mg/L.

Enhancement of nitrate in plot 1 occurred at day 27th, 33rd, 51st and 57th. Meanwhile in plot 4 increases was occurred at day 27th, 33rd, 39th, 51st, 57th and 60th. Furthermore in plot 7 increases occurred at day 27th, 36th, 45th, 51st, 57th and 60th. Significant increase in plot 1 occurred at day 57th to be 12 mg/L; in plot 4 occurred at day 51st and 60th to be 12 mg/L and 15 mg/L; and in plot 7 occurred at day 60th to be 45 mg/L. the deprivation of nitrate in plot 1 occurred at day 30th and 54th. In plot 4 decreases occurred at day 30th, 42nd and 54th. In plot 7 deprivation occurred at day 33rd, 39th and 48th. Significant deprivation of nitrate only occurred in plot 4 at day 54th to be 10 mg/L.

Nitrate is the final product of the biochemistry oxidization process in the water. Nitrate concentrations in an aquatic system is being controlled in the nitrification process. Nitrate comes from fertilizer residual, leftover feed, and free nitrogen binding from the air by microorganisms, as well as land stream to enter the sea. Nitrate formation will be smooth when bacteria work to recast nitrite [10].

Enhancement of phosphate in plot 1 occurred at day 27th, 30th, 36th, 42nd, 45th, 48th and 54th. Meanwhile in plot 4 occurred at day 27th, 33rd, 36th, 42nd, 45th, 57th and 60th. Then in plot 7 occurred at day 27th, 33rd, 36th, 39th, 45th, 51st, 57th and 60th. Significant enhancement in plot 1 occurred at day 54th to be 1 mg/L; in plot 4 occurred at day 42nd to be 1.35 mg/L and in plot 7 occurred at day 51st to be 1.75 mg/L. Phosphate deprivation in plot 1 occurred at day 60th, plot 4 occurred at day 30th, 39th and 48th, and plot 7 occurred at day 30th, 48th and 54th. Significant deprivation occurred in plot 4 at day 30th to be 1.2 mg/L and at plot 7 at day 57th to be 1.75 mg/L.

The phosphor is in the dissolved inorganic form (orthophosphate), dissolved organic and phosphate particles [11] mentioned that normally, phytoplankton able to directly assimilate dissolved inorganic and sometimes use dissolved organic phosphor. The phosphor is applied in energy transfer in phytoplankton's cells from ADP to be ATP.

Identification of Blue green algae during the research resulted: Anabaena sp., Chroccocus sp., Gomphosphaeria sp., Microcystis sp., Oscillatoria sp., and Spirulina sp. Quantity of identifiying phytoplankton based on genus resulted one class of Cyanophyceae.

Density enhancement of Blue green algae in plot 1 occurred at day 30th, 36th, 42nd, 48th and 54th. The most significant increase in plot 1 occurred at day 54 with to be 30.5X104 cells/ml. In plot 4 most increase density occurred at day 30th, 33rd, 39th, 42nd, 45th, 51st and 60th, with the most significant increase at day 51th to be $58.25X10^4$ cells/ml. Meanwhile in plot 7 the density increase was occurring at day 27th, 30th, 39th, 42nd, 45th, 48th, 51st and 57th. The most significant increase in plot 7 occurred at day 57th to be $21X10^4$ cells/ml.

Decrease of Blue green algae density in plot 1 occurred at day 27th, 33rd, 39th, 45th, 51st, 57th and 60th. The most significant decrease in plot 1 occurred at day 60th to be $21X10^4$ cells/ml. meanwhile plot undergoes density decrease at day 27th, 36th, 48th, 54th and 57th, and the most significant decrease occurred at day 57 to be $60X10^4$ cells/ml. Furthermore, in plot 7 density decrease occurred at day 33rd, 36th, 54th and 60th. The most significant decrease in plot 7 occurred on day 54 to be $22.75X10^4$ cells/ml.

[12] mentioned that enhancement of nitrate can bring up the domination of diatom because diatom need nitrate for cell division, while Blue green algae is more suitable to be in condition of high level of ammonium and low level of nitrate. Nitrate is the substance being used by diatoms, if nitrate is abundant then diatom will be abundant as well. The condition of the limited level of nitrate there will be less domination of diatom, because less nitrate to be utilized by diatom. [5] mentioned that Blue green algae is having an important role as producer of nitrogen in water, then Blue green algae able to survive in conditions of low level nitrate.

## 5. Conclusion

Based on the results of data analysis and discussion of the dynamic relationship of the N: P ratio of the abundance of green blue algae, it can be concluded that the value of the N: P ratio affects the composition of phytoplankton class in aquaculture waters. The N: P ratio affects the abundance of phytoplankton, but the levels and dynamics of ammonium, nitrite, nitrate and phosphate which specifically influence the abundance of green algae blue in the waters. High ammonium conditions and low nitrate can cause an abundance of green blue algae to increase. Water quality parameters as a supporting factor also affect the life of Blue Green Algae.

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Lampiran 3. Letter of Acceptance IFS 2018, Certificate of Participation as Oral Presentation dan Draft Artikel untuk Publikasi

MOE 0521.2.05/844

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July 31, 2018

**LETTER OF ACCEPTANCE**

**Dear** Endang Dewi Masithah, Daruti Dinda Nindarwi, Ayu Lana Nafisyah and Kazuhiko Koike

Faculty of Science and Technology, Prince of Songkla University (PSU), together with the ASEAN Fisheries Education Network (ASEAN-FEN) is holding the 8th International Fisheries Symposium (IFS2018) with the theme "Sustainable Fisheries and Aquaculture for the benefits of Mankind" which will be held during November 18-21, 2018 at Hansa JB Hotel, Hatyai, Thailand.

On behalf of PSU, ASEAN-FEN and the organizing committee, we are glad to inform you that your abstract has been **ACCEPTED** as detailed follows:

**Title** : Microcystin Toxin Production of Several Blue Green Algae Species from Banyuwangi, Indonesia, in Different Salinity Culture
**Type** : Oral Presentation

For more information on registration, program and related activities, kindly visit our website at http://ifs2018.sat.psu.ac.th. If you require additional documents for visa, please contact us at ifs2018thailand@gmail.com.

Please be informed that the deadline of full paper submission is on December 31, 2018.
30 selected papers will be published in Songklanakarin Journal of Science and Technology (www.sjst.psu.ac.th). Others will be published as proceeding for IFS2018.

We are looking forward to welcoming you in Hatyai, Thailand.

Sincerely yours,

Assoc. Prof. Dr. Sukree Hajisamae
Chairperson of the Organizing Committee,
Dean of Faculty of Science and Technology
Prince of Songkla University

PRINCE OF SONGKLA UNIVERSITY

ASEAN Fisheries Education Network

# CERTIFICATE OF PARTICIPATION

**This is to certify that**

## ENDANG DEWI MASITHAH

has actively participated and given ***oral presentation*** in

**THE 8TH INTERNATIONAL FISHERIES SYMPOSIUM - IFS2018**

***"Sustainable Fisheries and Aquaculture for the Benefits of Mankind"***

**HELD IN HATYAI, THAILAND, FROM NOVEMBER 18TH – 21ST, 2018**

(ASSOC. PROF. DR. SUKREE HAJISAMAE)
CHAIRMAN OF THE ORGANIZING COMMITTEE
DEAN, FACULTY OF SCIENCE AND TECHNOLOGY,
PRINCE OF SONGKLA UNIVERSITY, PATTANI CAMPUS

# Microcystin Toxin Production of Several Blue Green Algae Species from Banyuwangi, Indonesia, in Different Salinity Culture

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**Abstract.** Cyanobacterial blooms are widespread in temperate and tropical freshwater systems. To find out the relationship between microcystins (MCs) and salinity requires testing that can determine the production of microcystin (MCs) toxin from several Cyanobacteria species cultured in different salinity media. Water sampling on the ponds taken from the intensive culture in Banyuwangi and on fresh water taken from traditional culture in Lamongan. Cyanobateria isolation species were obtained from the School of Biosphere Science, Hiroshima University. The result microcystin production of Cyanobacateria is competition with another Phytoplankton, saline stress caused a decrease in the transcript level of mcyD (one of gene involved in microcystin synthesis), the increase of salinity caused decrease of microcystis growth but promote to microcystin release in the waters.

*Keywords* : Microcystins: Cyanobacteria: Environmental ; Salinity

## *1. Introduction*

Indonesia is a tropical country that has two seasons, and has a long enough time to be exposed to sunlight because it is on the equator. has a tropical climate and is on the equator to make Indonesia as one of the countries that has a high diversity of phytoplankton. Cyanobacterial blooms are widespread in temperate and tropical freshwater systems.

Blooms are promoted by nutrient-replete, warm, slow-moving or stagnant waters, which allow *Microcystis* to proliferate and form a thick green scum on surface waters (Otten *et al*., 2012). Many *Microcystis* strains produce microcystins (MCs), a prevalent and widespread cyanotoxin with more than 100 variants currently reported (Chorus and Bartram, 1999; Puddick, 2013; Corbel *et al*., 2014). MCs are present in a broad range of aquatic systems (Chorus and Bartram, 1999; Song *et al*., 2007; Poste *et al*., 2011; Jia *et al*., 2014), and are potent hepatotoxins and potential tumor promoters (Falconer and Humpage, 1996; de Figueiredo *et al*., 2004; Chen *et al*., 2009).

Blooming cyanobacteria have not reached a significant density to produce microcystines (MCs) which can affect other organisms in dangerous concentrations. There is currently not much research that addresses dangerous algae including microcystines (MCs). Actually in Aquaculture today, Cyanobacteria blooms also occur in salty

ponds so that it can make shrimp in particular become easily attacked by WSSV due to the occurrence of Cyanobacteria blooms in salty ponds (intensive shrimp farming) which then produce microcystin (MCs).

Laboratory studies indicate that MCs production in *Microcystis* are affected by various environmental factors such as light, temperature, nutrients, trace elements, salinity, pH, nutrient contents in cells and salinity (Lee et al. 2000; Long et al. 2001; Jahnichen et al. 2007; Li et al. 2007). So it is necessary to do research on microcystin production in relation to salinity.

In the present study, the objectives of the microcystin (MCs) toxin production of several Cyanobacteria species that are cultured in different salinity mediums can be used as a reference for further research on aquaculturist microcystin (MCs) toxin production and to guide in managing water quality to suppress microcystin (MCs) production in ponds so that the Shrimp is not suspectible to WSSV.

## *2. Materials and Methods*

This study was carried out at Banyuwangi city and Lamongan city. Especially for water sampling on salty ponds taken from the intensive culture in Banyuwangi and on fresh water ponds taken from traditional culture in Lamongan. This study was conducted for nine month, namely on March to December 2017. Cyanobateria species isolation obtained from the School of Biosphere Science, Hiroshima University. Culture of Cyanobacteria was conducted at the Faculty of Fisheries and Marine, Universitas Airlangga. The microcystin (MCs) measurement was carried out by testing ELIZA at the Tropical Diseases Institute, Universitas Airlangga.

## *3. Results and discission*

### *3.1 Result*

Microcystin (MCs) Concentration in Traditional Pond (Fresh Water) and Intensive Pond

**Table 1. Microcystines (MCs) Concentration in Traditional Pond (Fresh Water)**

| No | Pond | Species of Cyanobacteria | Cyanobacteria Density ($10^4$cel/ml) | Microcystin Concentration (ppb) |
|---|---|---|---|---|
| 1 | Pond 1 | *Microcystis* | 8 | 1,0925 |
| | | *Oscilatoria* | 5 | |
| 2 | Pond 2 | *Microcystis* | 8 | 1,2035 |
| | | *Oscilatoria* | 4 | |
| 3 | Pond 4 | *Microcystis* | 6 | 1,4975 |
| | | *Oscilatoria* | 4 | |
| | | *Anabaena* | 2 | |
| 4 | Pond 5 | *Microcystis* | 6 | 1,1650 |
| | | *Oscilatoria* | 5 | |

**Table 2. Microcystines (MCs) Concentration in Intensive Pond**

| Pond | Species of Cyanobacteria | Cyanobacteria density ($10^4$cel/ml) | Microcystin Concentration (ppb) | | |
|---|---|---|---|---|---|
| | | | Water | Gill | Hepato |
| I.1 | *Oscillatoria sp.* | 1 | 2,113 | 1,181 | 2,817 |
| | *Microcystis sp.* | 7 | | | |
| | *Anabaena sp.* | 0,25 | | | |
| | *Chroococcus sp.* | 0,25 | | | |
| I.2 | *Oscillatoria sp.* | 1,5 | 1,310 | 0,021 | 2,639 |
| | *Microcystis sp.* | 7,5 | | | |
| | *Anabaena sp.* | 0,5 | | | |

**Table 3. Water Quality of Intensive Ponds**

| Ponds | Salinity (ppt) | Alkalinity (ppm) | Nitrite (ppm) | Nitrate (ppm) | Ammo-nium (ppm) | Phospate (ppm) | N/P Ratio |
|---|---|---|---|---|---|---|---|
| I.1 | 26 | 170 | 2.5-10 | 5 | 0.2-2.3 | 0.25-1.25 | 6.7-28.9 |
| I.2 | 20 | 165 | 0.3-5 | 3 | 0.2-3.9 | 0.3-1.75 | 2.4-28.4 |

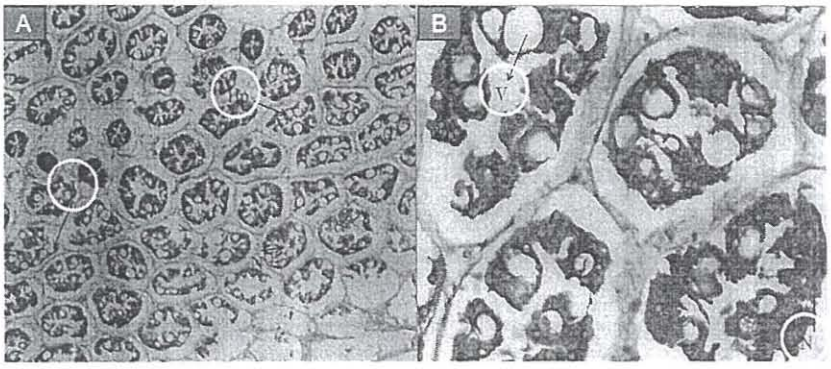


**Figure 1.** Histopathology of Shrimp Hepatopancreas in pond I.1
a. V = Vacuolization; Hp = Hyperplasia; N =Necrosis (HE, 100x)
b. V = Vacuolization; N = Necrosis (HE, 400x)

Figure 1 shows the results of histopathology of shrimp hepatopancreas who are exposed to microcystines (MCs) so that in picture A we can see it vacuolization, hyperplasia, and necrosis and than in picture B we can see it vacuolization, and necrosis in pond I.1.

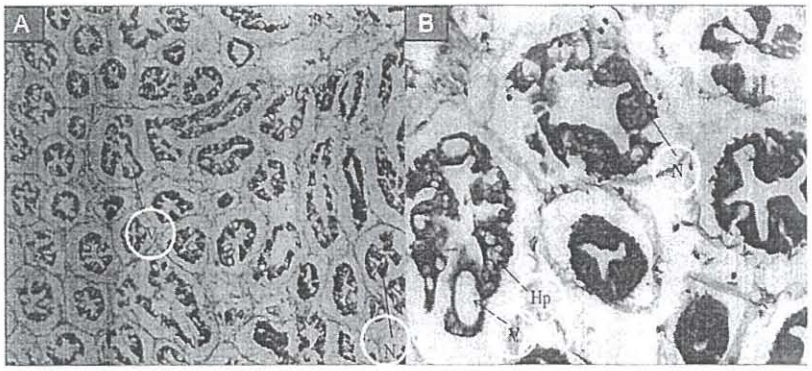


**Figure 2.** Histopathology of Shrimp Hepatopancreas in pond I.2
a. V = Vacuolization: N = Necrosis (HE, 100x)
b. V = Vacuolization: Hp = Hyperplasia; N = Necrosis (HE, 400x)

Figure 2 shows the results of histopathology of shrimp hepatopancreas who are exposed to microcystines (MCs) so that in picture A we can see it vacuolization, and necrosis and than in picture B we can see it vacuolization, hyperplasia, and necrosis in pond I.2.

Table 4. Laboratory Culture Microcystines (MCs) Production in Various Salinity Medium

| No | Spesies | Salinity (ppt) | Plankton density ($10^4$cel/ml ) | Microcystin Concentration ( ppb ) |
|---|---|---|---|---|
| 1 | *Microcystis* sp | 9 | 7 | 0,7709 – 1,2654 |
| | | 20 | 5,5 | 1,0555 – 1,9415 |
| | | 33 | 5 | 1,8910 – 2,3653 |
| 2 | *Anabaena* sp | 20 | 6 | 0,6703 – 1,7396 |
| | | 33 | 4 | 0,7504 – 2,718 |
| 3 | Green Chain | 20 | 5 | 0,9324 – 1,2856 |
| 4 | *Sprulina* sp. | 9 | 8 | ± 0,01 |
| | | 20 | 4 | ± 0,01 |
| | | 33 | 4 | ± 0,01 |

### 3.2 Discussion of Results

Based on table 4, we know about something, namely salinity affects the density of algae and also the production of microcystines (MCs) toxin, this can be seen from the laboratory scale of cyanobacteria from *Microcystis* sp. the higher salinity, the plankton density will decrease but not so with microcystines (MCs) concentration which tends to increase at high salinity. Same as *Anabaena* sp. and *Spirulina* sp. which tends to decrease in density if at high salinity but is inversely proportional to the microcystines (MCs) concentration which tends to increase or stagnant. And this is in line with what was stated by Jia *et al.* (2018), namely that the results showed that microcystines MCs concentrations were not always decided by the abundance of cyanobacteria, and there were differences in the ability of cyanobacteria to produce poisons in different salinity.

So that we can assume that when we cultivate Cyanobacteria in fresh water (low salinity) conditions will be increase the cyanobacteria density when we cultivate it in Salty water (high salinity) conditions. But to reduce microcystines (MCs) concentration we can change its salinity to be lower or cultivate it in a fresh water state than salty water which can make high microcystines (MCs) concentration.

## 4. Conclusion

Salinity is thought to have an effect on microcystines (MCs) production by blue green algae. Further research is needed on several factors that influence microcystines (MCs) production in addition to salinity.

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Lampiran 4. Draft Artikel untuk Publikasi pada Jurnal Terindeks Scopus

Judul Artikel :

Microcystin Concentration in Vannamei (*Littopenaeus vannamei*) Intensive Ponds in Banyuwangi, East Java

# MICROCYSTIN CONCENTRATION IN VANNAMEI ((*Litopenaeus vannamei*) INTENSIVE PONDS IN BANYUWANGI, EAST JAVA

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**Abstract**

Phytoplankton from the Cyanophyceae class such as cyanobacteria are known to produce toxins (cyanotoxins) which are detrimental in aquaculture. This toxin includes hepatotoxins such as microcystin. Shrimp that swallow cyanotoxins were reported to induce hemocytic enteritis, a disease in which the middle epithelial lining is damaged and a healthy mucous layer is replaced by necrotic and inflammatory cells in the hemocyte layer. So than that, a study was conducted to determine the concentration of microcystin in intensive shrimp vaname ponds (*Litopenaeus vannamei*). This study used survey method to describe existing phenomena, which take place at this time or in the past. The results showed that the dominance of cyanobacteria affected the concentration of microcystin toxins in the observed samples. The highest sequence of microcystin concentrations was found in the hepatopancreas, followed by water in the pond of shrimp and gills having the lowest concentration of microcystin. The accumulation of microcystin toxins in the gill organs and vaname shrimp hepatopankreas also showed histopathological changes

Keywords : Cyanobacteria, Microcystin, Gill Histopatology, Hepatopancreas Histopatology, *Litopenaeus vannamei*

## Introduction

Probiotic applications into an intensive ponds of fish and shrimp are widely used to improve water quality and increase growth. As stated by Tangko *et al.* (2007) that in the field of aquaculture, the use of probiotics aims to maintain microbial balance and control pathogens in the digestive tract of cultivated organisms, as well as the aquatic environment through the process of biodegradation.

Sudiana *et al.* (2002) describe the types of probiotic bacteria that play a role in the decomposition of organic matter including Pseudomonas, Bacillus and Enterobacter. Bacillus bacteria are phosphate decomposing bacteria that are commonly found in sediments. Phosphate decomposing bacteria play a role in supplying phosphate compounds to the ecosystem. In dead bacterial cells, the P element in the form of PO4 (phosphate) will undergo a change to organic P, in this condition a solution of bacterial cells rich in organic P. Makmur *et al.* (2012) explained that the composition between nutrient components, namely the ratio of N to P which is often referred to as redfield ratio, will affect the abundance of certain types of phytoplankton. N: P ratio above 20 will be more dominated by diatome phytoplankton, while N: P ratio with a value of around 10 will be dominated by green phytoplankton (chlorella) while N: P ratio under 10 is a conducive environment for phytoplankton of Blue type Green Algae (BGA) including cyanobacteria (Lagus, 2009).

Some types of phytoplankton from the Cyanophyceae class such as cyanobacteria are known to produce toxins (cyanotoxins) which are detrimental in aquaculture activities (Hoek *et al.*, 1995). This toxin includes hepatotoxins such as microcystin (Downing *et al.*, 2015). Shrimp that swallow cyanotoxin are reported to induce hemocytic enteritis, a

disease in which the middle epithelial lining of the intestine is damaged and a healthy mucous layer is replaced by necrotic and inflammatory cells in the hemocyte layer (Lightner, 1978). The acute effects of these toxins also contribute to the long-term productivity of shrimp farming through inhibition of growth to death (Kankaanpää *et al.*, 2005).

Based on this background, research is needed to determine the concentration of microcystin in intensive shrimp vaname ponds (*Litopenaeus vannamei*). The study was conducted by taking samples of vaname shrimp from intensive ponds which using probiotics on their maintenance media, then observing the diversity and density of phytoplankton and microcystin content, also determine the content of microcystin and making histopathological examination from gill organs and hepatopancreas.

## Material And Methods

This study used plankton net, container (bucket), haemocytometer, drop pipette, microscope, glass object, glass cover, sample bottle, burette, statif, clamp, 100 ml measuring cup, and Sectio instruments (scissors, scalpel, and tweezers), ELISA equipment, histopathology equipment.

The samples used were vaname shrimp (*Litopenaeus vannamei*) within DOC 94 and water quality which taken from two vaname shrimp ponds (Station B-6 and station B-7). The gill and hepatopancreas organ were prepared to follow the protocol stated on the Microcystin ELISA test kit for measuring the concentration of microcystin using the ELISA test and histopathology examination

### Identification of phytoplankton

Phytoplankton observations included identification of diversity and density to determine the dominance of cyanobacteria in intensive vaname shrimp ponds. The obtained water samples were labeled and phytoplankton observations were immediately observed using a 100-400x binocular microscope magnification (Nontji, 2008).

### Gills and Hepatopancreas Examination

The gills and hepatopancreas that have been obtained were washed using a solution of Phosphate Buffered Saline (PBS pH 7.4). The gills and hepatopancreas were weighed 0.1 grams each then put in a 1.5 ml tube and 1 ml of extraction buffer solution was added.

The organ and extraction buffer solution in the tube were homogenized by cutting it to shreds, then vortexed for 20 seconds and centrifuging at 3000 rpm for 3 minutes at room temperature (25°C). Supernatants were taken as much as 100 µl carefully using yellow tip and transferred into a 1.5 ml empty tube, then added 900 µl of sterile distilled water (1:10 dilution), the result of this dilution to be used for ELISA .

### Analysis of Microcystin Concentration

The microcystin concentration was analyzed using a Microcystin ELISA kit

### Histopathology Examination

The procedure for making histopathological preparations in this study was carried out based on the procedures of Austin and Austin (1989) which included: fixation, dehydration and clearing, planting in paraffin (embedding), cutting, coloring, closure and observation. Shrimp organs used for the histopathological examinations were gills and hepatopancreas.

## Result

### Dominance of Cyanobacteria

The observation of cyanobacteria dominance in station B-6 and B-7 showed that cyanobacteria dominated the B-6 as much as 38.6%, respectively The value of cyanobacteria dominance and the average concentration of microcystin in the observed station shown in table 1 below:

Tabel 1. Dominance of Cyanobacteria

| Cyanobacteria | Cyanobacteria Dominance |
|---|---|

| | Station B-6 | Station B-7 |
|---|---|---|
| *Microcystis* sp. | 18,1% | 10,0% |
| *Anabaena* sp. | 0,7% | 0,4% |
| *Oscillatoria* sp. | 11,6% | 6,9% |
| *Spirulina* sp. | 4,5% | 0,5% |
| *Gomphosphaeria* sp. | 1,4% | 0,7% |
| *Chroococcus* sp. | 2,3% | 4,9% |
| Total | 38,6% | 23,4% |

**Microcystin Concentration**

The results of the concentration of microcystin in intensive ponds of vaname shrimp are fully presented in Table 2 below:

Table 2. Data on microcystin concentration in intensive ponds of vaname shrimp

| Station | Time | Microcystin Concentration ppb (ng/ml) |
|---|---|---|
| B-6 | 05.00 | 2,113 |
| | 13.00 | 1,3108 |
| B-7 | 05.00 | 1,3108 |
| | 13.00 | 0,5742 |

**Microcystin Concentration in Gills and Hepatopancreas of Vaname Shrimp**

The results of microcystin concentrations are presented in Table 3 below:

Table 3. Data on microcystin concentration in gill and vaname shrimp hepatopankreas

| Station | Microcystin in gills ppb (ng/gr) | Microcystin in Hepatopankreas ppb (ng/gr) |
|---|---|---|
| B-6 | 0,6095 | 3,6973 |
| B-7 | 0,01 | 2,2896 |
| B-6 | 0,6852 | 1,5378 |
| B-7 | 0,01 | 2,1635 |
| B-6 | 1,3108 | 3,9596 |
| B-7 | 0,01 | 2,1079 |
| B-6 | 2,1181 | 2,0726 |
| B-7 | 0,0545 | 3,9949 |

**Histopathology Observations**

**Gill histopatology of vannamei shrimp in Station B-6**

Observation of histopathological of vaname shrimp gills showed hyperplasia which was found at the bottom of the lamella as seen in the following figure:

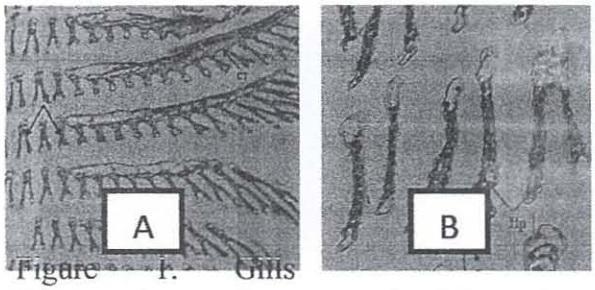


Figure 1. Gills Histopatology of vannamei shrimp in station B-6 (A = L = Lamellae; CT = Connective tissues; (HE, 100x), B = Hp = Hiperplasia (HE, 200x)

**Gills histopathology of vannamei shrimp in station B7**

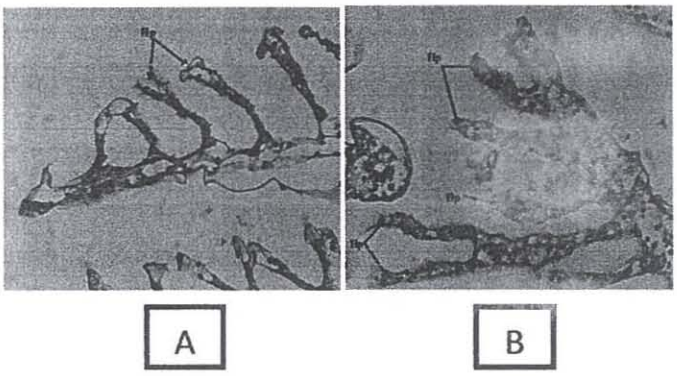


Figure 2. Gills Histopatology of vannamei shrimp in station B-7 (A : Hp = Hiperplasia (HE, 200x), B : Hp = Hiperplasia (HE, 200x)

**Histopatology of vannamei shrimp Hepatopankreas in station B6**

Observation of histopathological hepatopancreatic organ were vacuolized. Histopathology of vannamei shrimp

hepatopankreas in station B-6 is presented in figure 3 below:

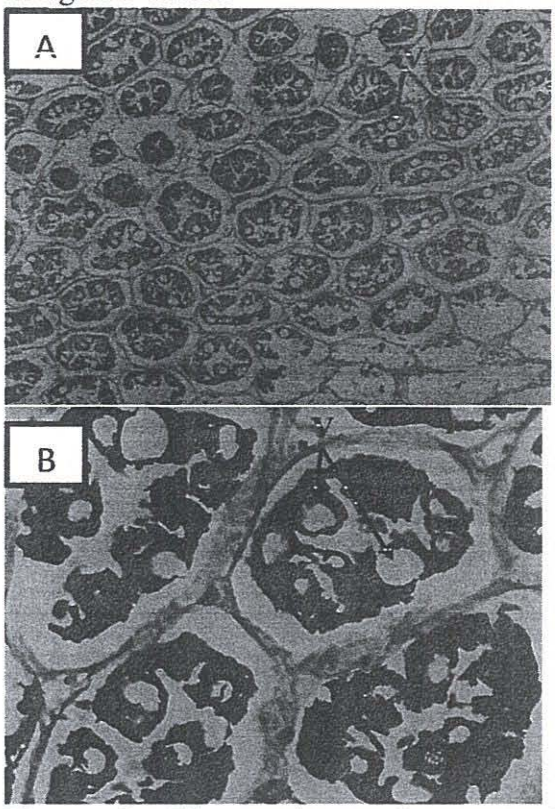


Figure 3. Histopatology of vannamei shrimp Hepatopankreas in station 6 (A: V = Vaccuolization (HE, 100x), B : V = Vakccuolization (HE, 400x)

**Histopatology of vannamei shrimp Hepatopankreas Udang Vaname in station B7**

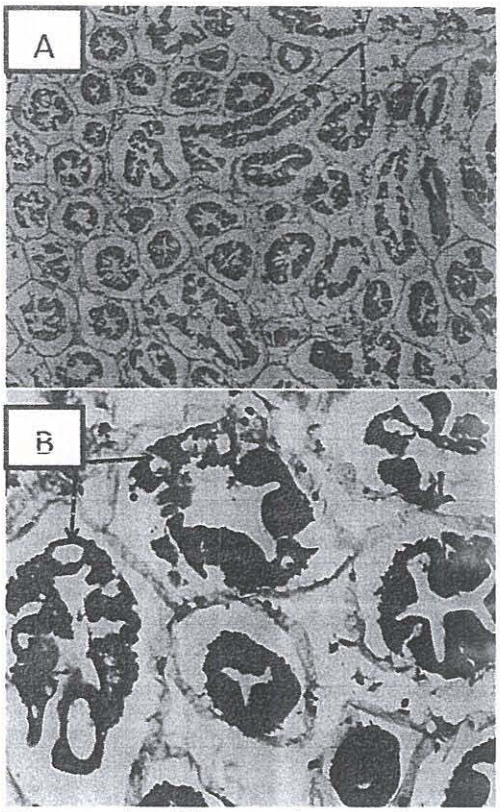


Figure 4. Histopatology of vannamei shrimp Hepatopankreas in station B-7 (A : V = Vaccuolization (HE, 100x), B : V = Vaccuolization (HE, 400x)

## DISCUSSION

The results of phytoplankton observations on the station B-6 were found in six genera of cyanobacterias, were *Microcystis* sp. (18%), *Anabaena* sp. (1%), *Oscillatoria* sp. (12%), *Spirulina* sp. (5%), *Gomphosphaeria* sp. (1%) and *Chroococcus* sp. (2%). The overall dominance of cyanobacteria in station B-6 were 39%. This result showed that station B-6 has a high dominance value of cyanobacteria. The predominant type of cyanobacteria is *Microcystis* sp. and *Oscillatoria* sp. *Microcystis* sp. and *Oscillatoria* sp. is known to be a cyanobacteria that produces hepatotoxin (microcystin). The diversity and dominance were influenced by many factors such as water quality, aquatic nutrients and environmental factors (Goldman and Horne, 1983).

The results of phytoplankton observations in the station B-7 were found

in six genera of cyanobacterias, namely *Microcystis* sp. (10%), *Anabaena* sp. (0%), *Oscillatoria* sp. (7%), *Spirulina* sp. (1%), *Gomphosphaeria* sp. (1%) and *Chroococcus* sp. (5%). The overall dominance of cyanobacteria in the B-7 plot is 24%. This shows that the dominance of cyanobacteria in station B-7 was lower than in station B-6. The highest dominance of cyanobacteria in station B-7 was *Microcystis* sp. (10%) which is also known as cyanobacteria which produce hepatotoxin (microcystin).

Some of these species found in this research are known to produce microcystin toxins. Microcystin is a group of cyclic poisons produced by brackish and freshwater cyanobacteria from the genera Anabaena, Anabaenopsis, Chroococcus, Microcystis, Nostoc, Planktothrix and Oscillatoria (Chorus, 2001; Prihantini *et al*., 2006; Pearson *et al*., 2010) . This toxin is a secondary metabolite product that accumulates in the cytoplasm in certain situations (Paerl and Millie, 1996). Microcystin is stable in water and is hepatotoxic (Romanowska-Duda *et al*., 2002).

Based on the results of the measurement of microcystin concentrations in the gill and vaname shrimp hepatopancreas and shrimp ponds, the three types of samples showed different results of microcystin concentrations. The highest concentration of microcystin was obtained from the sample of vanamei shrimp hepatopancreas, which was as much as 3.9949 ppb (ng / gr) with an average of 2.7279 ppb (ng / gr). This is consistent with the statement that microcystin is a hepatotoxin and liver poison is the main target of microcystin (WHO, 2003).

The lowest concentration of microcystin was obtained from the sample of vanamei shrimp gills, which was as much as 0.01 ppb (ng / gr) with an average of 0.0211 ppb (ng / gr). The low concentration of microcystin in the vaname shrimp gills were suspected because the exposure of toxins to the gills only occurs passively, as through water contact with the gill epithelium (Malbrouck and Kestemont, 2006).

Whereas in the water sample of vaname shrimp ponds, the lowest microcystin concentration was 0.5742 ppb (ng / mL) and the highest concentration of microcystin was 2.1130 ppb (ng / mL) with an average of 1.2121 ppb (ng / mL). ) The concentration of microcystin in pond water samples is quite high when compared to the research conducted by Covarrubias *et al.* (2016), which obtained the results of microcystin concentration in treated water as much as 0.78 ppb (µg / L). The concentration of microcystin in pond water is higher compared to the concentration of microcystin in gills, this could because cyanobacteria toxins (microcystin) could be released into the water as long as they experience aging to lysis, these toxins can dissolve in water (Drobac *et al*., 2016) and very stable in water, resistant to extreme pH and temperatures in 300°C (WHO, 2003).

Among cyanotoxin, microcystin with a hepatotoxic promoter which can also cause liver tumors is considered one of the most dangerous groups of toxins (Li *et al*., 2017). Microcystin exposure also occurs passively, namely through direct contact of the gill epithelium with surrounding water containing the toxin (Malbrouck and Kestemont, 2006). Therefore, in this research, histopathological examination of gill organs and hepatopankreas (*Litopenaeus vannamei*) were made to be observed microscopically.

Based on the observation of histopathology of vaname shrimp gill organs, hyperplasia appears with percent damage of 22.5% from station B-6, and 20% in the sample from station B-7. Several pathological changes that were found in the observation of vaname gill organ histopathology preparations were hyperplasia. Mulyani *et al*., (2014), stated that hyperplasia can occur due to chemical

stimuli from pollutants, environmental pollution, parasitic and bacterial infections. Hyperplasia in addition to suppressing the capillaries of blood vessels in cells will also require an increase in blood supply to newly formed tissue. In chronic conditions once the cell condition is not normal again but will stick together (Hibiya, 1995).

On the observations of histopathologica of vaname shrimp hepatopankreas organ, there was vacuolization, which is the formation of space in cells containing fat due to cell degeneration characterized by the appearance of vacuoles in the hepatopancreas tubule. Vacuolization is characterized by tubular epithelial cells seen under the microscope losing their cell contents or empty (Soegianto, *et al.*, 2004). Percentage of vacuolation damage in vaname shrimp hepatopankreas samples obtained from Sation B-6 was 61.25%, while percent of vacuolated damage in vaname shrimp hepatopankreas samples obtained from Sation B-7 was 57.5%,

Dawson (1998) reported histopathological changes in hepatocytes after exposure to microcystin, due to the destruction of the cytoskeleton caused by increased levels of protein phosphorylation. In the research of Covarrubias *et al.* (2016), hepatopancreas from postlarva shrimp exposed to Microcystis aeruginosa (cyanotoxin) shows severe haemocytic infiltration, some tubules undergo necrosis and there is melanization in tissues. Melanization is one of the immune responses in shrimp. Crustaceans are thought to carry a simple and primitive immune system, where cellular reactions to defense are often followed by a melanization process (Soderhall and Cerenius, 1992).

Gills in crustaceans play a role in the process of respiration, acid-base balance, ionic and osmotic regulation because of the presence of branchial epithelium tissue which is the site of active transport of important ions between organisms and the environment (Soegianto *et al.*, 1999), and plays an important role in toxicology crustaceans (Morales-Covarrubias *et al.*, 2016). While the hepatopankreas is the most important organ in shrimp, because these organs function like the liver and pancreas in mammals (Soegianto *et al.*, 1999). The high damage to the gill and hepatopancreas structures will affect the process of enzyme metabolism and osmoregulation in shrimp. Besides damage to cells caused by poisoning or other factors, for example stress conditions, can increase sensitivity to viral and bacterial infections (Snieszko, 1974). This can quickly increase the risk of death in shrimp (Soegianto et al., 2004).

This is consistent with the statement Sousa and Petriella (2007) that the hepatopancreas is very sensitive to the effects of pollution, so this organ is often used to determine the effects of various toxicants. Histopathological changes can provide information on stress levels, susceptibility and adaptability to the ability of an organism to deal with stress, as well as in the hepatopankreas organ of vaname shrimp.

## Conclusion

From this research we conclude that the types of cyanobacteria found in vannamei shrimp intensive ponds were *Microcystis* sp., *Anabaena* sp., *Oscillatoria* sp., *Spirulina* sp., *Gomphosphaeria* sp. and *Chroococcus* sp. Also from this research we know that the dominance of cyanobacteria affects the concentration of microcystin toxin in gill , hepatopankreas and the pond water.

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Lampiran 5. Letter of Invitation pada Academic Assimilation di UMT, Sertiicate as Presenter dan Foto Kegiatan


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: pro@umt.edu.my
**PUSAT PENGAJIAN SAINS PERIKANAN DAN AKUAKULTUR**


**Ref. : UMT/PPSPA/D/100-6/2 Jld. 1 (14)**
**Date : 14 November 2018**

Dr. Endang Dewi Masithah
Faculty of Fisheries and Marine
Universitas Airlangga (UNAIR)

Dear Dr. Endang,

**ACADEMIC ASSIMILATION BETWEEN SCHOOL OF FISHERIES AND AQUACULTURE SCIENCES, UMT AND FACULTY OF FISHERIES AND MARINE, UNAIR, 24 - 27TH NOVEMBER 2018**

The above matter refers.

2. In conjunction with the program, on behalf of the School of Fisheries and Aquaculture Sciences, it is our great honour to invite you to give a 20-minute talk on "**Microcystis as harmful algae**". The details are as follows:

Date : 25th November 2018
Time : 2.15 pm
Venue : Ibn Rushd Seminar Room

3. We believe that your contribution to this topic is unparalleled, and thus provides an opportunity for both institutions to share some current issues and discuss the topic related to the future of aquaculture and fisheries in our regions.

We look forward to your participation and thanking you in advance.

**"OCEAN OF DISCOVERIES FOR GLOBAL SUSTAINABILITY"**

Sincerely yours,

**PROF. DR. NAJIAH BINTI MUSA**
Dean
School of Fisheries and Aquaculture Sciences
Universiti Malaysia Terengganu
09-6685154
najiah@umt.edu.my

c.c. 1) Assoc. Prof. Dr Nadirah Musa, PIC MoU UMT-UNAIR
School of Fisheries and Aquaculture Sciences
2) File

UNIVERSITI MALAYSIA TERENGGANU

UMT

# Certificate of Recognition

Presented to

**DR. ENDANG DEWI MASITHAH**
**(Invited Speaker)**

For giving an informative and insightful talk on

**Microcystis as harmful algae**

in

**Academic Assimilation 2018**

between

**School of Fisheries and Aquaculture Sciences,**
**UNIVERSITI MALAYSIA TERENGGANU**

and

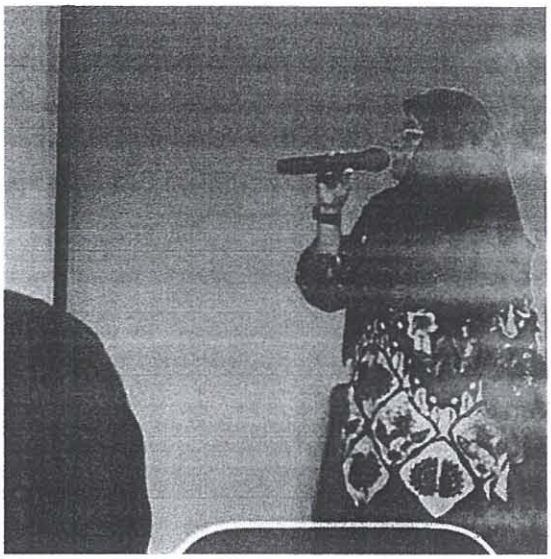
**Faculty of Fisheries and Marine,**
**AIRLANGGA UNIVERSITY**

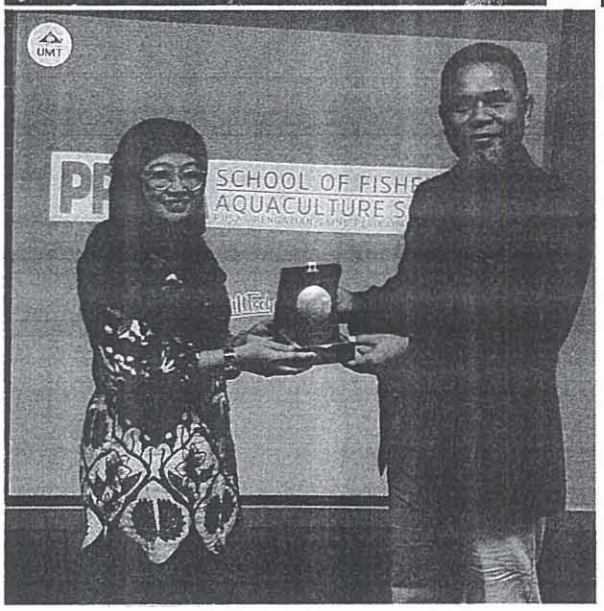
on

**25th November 2018**

**PROFESSOR DR. NAJIAH MUSA**
Dean
School of Fisheries and Aquaculture Sciences
Universiti Malaysia Terengganu
MALAYSIA

Foto Kegiatan Academic Assimilation di UMT Malaysia, 2018





Lampiran 6. Foto Sebagai Pembicara Seminaar Nasional Shrimp Club Indonesia Cabang Banyuwangi