Annex 7: Scientific Thinking and Species Biodiversity Index

By Dr. Chittima Aryuthaka

Annex 7

Self - introduction

name : Chittima Aryuthaka

education :

D.SC. in Marine Biology, Kyushu Univ

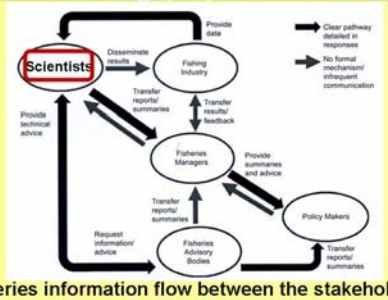
Thesis – Taxonomy and ecology on free – living marine nematodes in *Zostera marina* bed, Amakusa, Japan

Self - introduction

career: Associate professor
Department of Marine Science
Faculty of Fisheries Kasetsart Univ.

main teaching subjects:
Marine Ecology,
Marine Benthic Community,
Reproductive Strategies of Marine Benthc
Research methods in marine science

necessary of scientific information in



Fisheries information flow between the stakeholders (Soomai, 2010)

What is scientific procedure & why do we need to follow the procedure ?

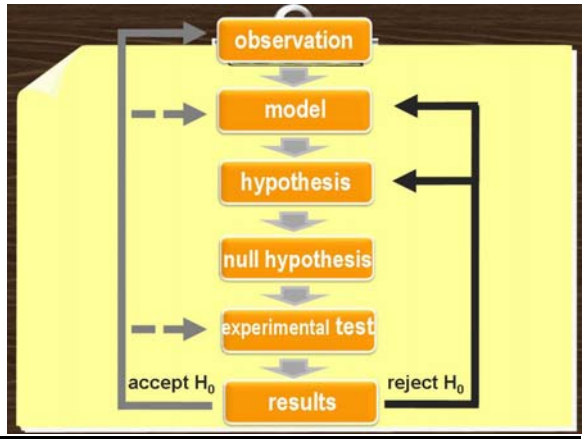
How do scientists define criteria for result decision ?

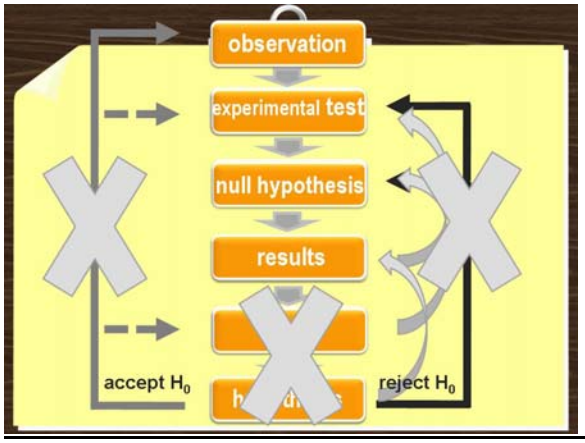
Statistical test

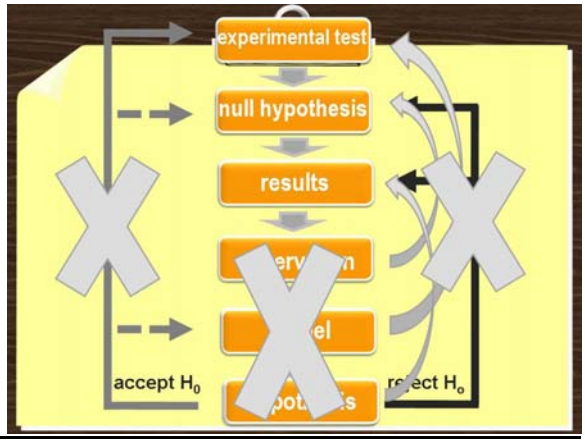
Analysis of Variance (ANOVA)

Biological diversity

Logical process in scientific research







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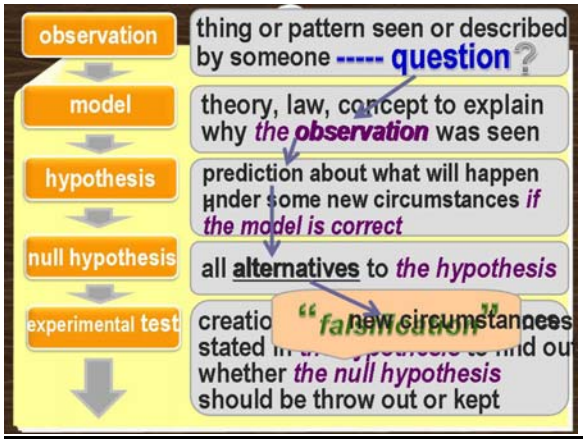
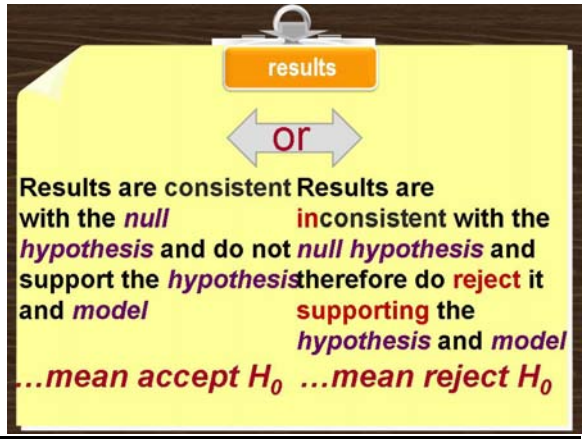
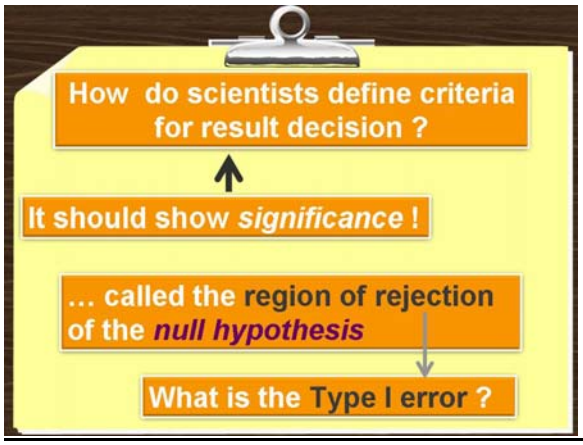
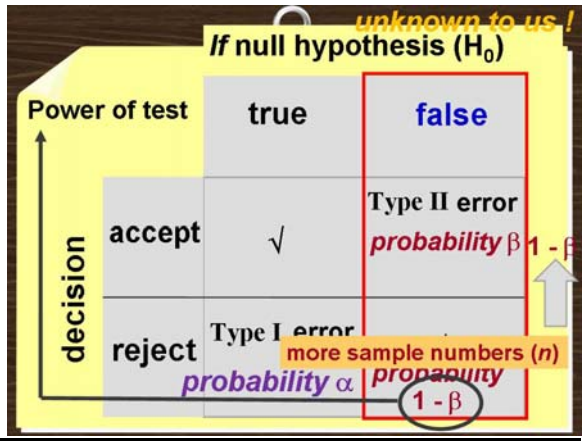
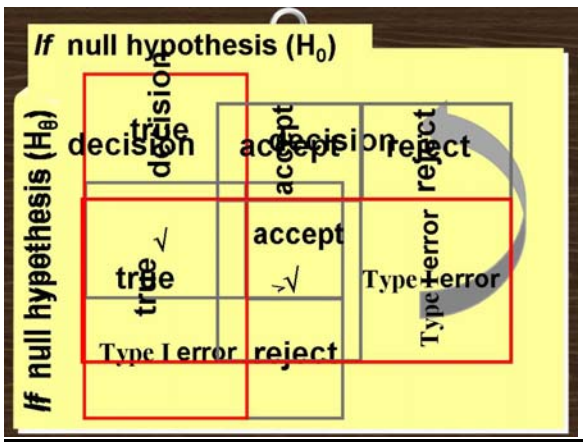
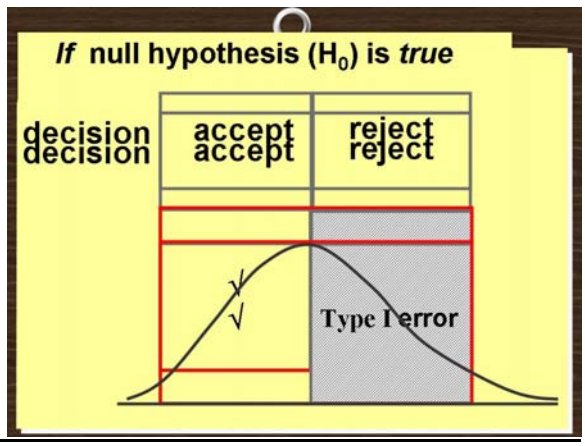

observation
thing or pattern seen or described by someone ----- question ?
model
theory, law, concept to explain why the observation was seen
hypothesis
prediction about what will happen under some new circumstances if the model is correct
null hypothesis
all alternatives to the hypothesis
experimental test
"falsification"
new circumstances
whether the null hypothesis should be throw out or kept

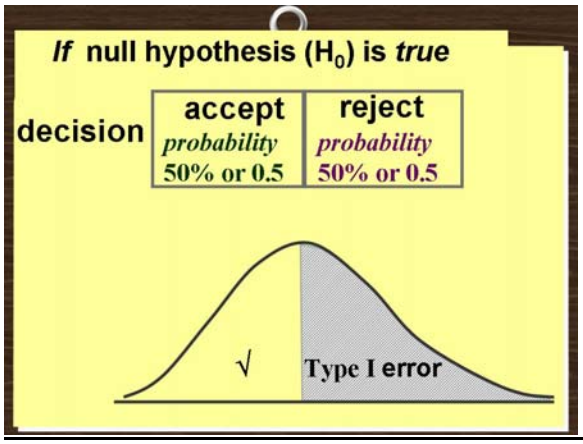

results
or
Results are consistent with the null hypothesis and do not support the hypothesis and model
Results are inconsistent with the null hypothesis and therefore do reject it supporting the hypothesis and model
...mean accept H_0
...mean reject H_0

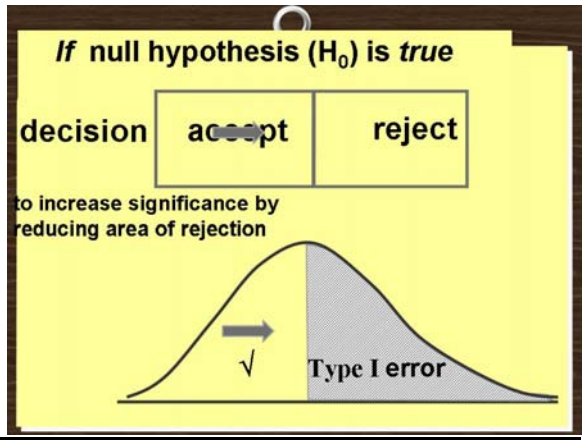

How do scientists define criteria for result decision ?
It should show significance !
... called the region of rejection of the null hypothesis
What is the Type I error ?


unknown to us !
If null hypothesis (H_0)
Power of test
true
false
decision
accept
reject
Type II error
probability β
1 - β
Type I error
probability α
more sample numbers (n)
1 - β


If null hypothesis (H_0)
If null hypothesis (H_0)
decision
true
accept
reject
√
Type I error


If null hypothesis (H_0) is true
decision
accept
reject
Type I error


If null hypothesis (H_0) is true
decision
accept
probability
50% or 0.5
reject
probability
50% or 0.5
√
Type I error


If null hypothesis (H_0) is true
decision
accept
reject
to increase significance by reducing area of rejection
√
Type I error

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If null hypothesis (H_0) is *true*

decision | accept | reject

probability 95% or 0.95

* * *highly significant*
α = 0.01
* *significant*
Type I error
α = 0.05

ANOVA : Analysis of Variance

F - value in *F* - distribution

accept *null hypothesis* (H_0)

* reject *null hypothesis* (H_0)

α = 0.05

F calculation

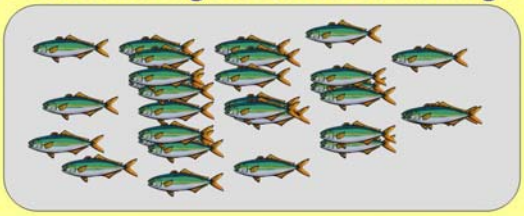
$F_{0.05}$ *F* calculation

F - value

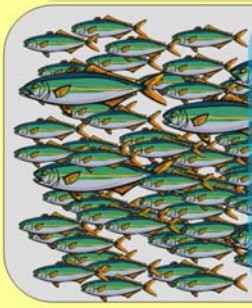
small large

Statistical tests

any of several tests of the statistical significance of findings



Statistical tests



fish population

size of [illegible] in the population

collecting fish samples and measure size

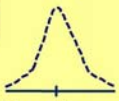
parameter

Numerical characteristic of a population computed using every element in the population. For example, the *mean* and the mode are parameters of a population.

variable

something that is changed or altered in an experiment. Having no fixed quantitative value measured from samples. For example, length, weight of fish, density etc.

parametric tests

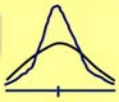


- *location parameter* (μ)

determines where the origin will be located. If μ is positive, the origin will be shifted to the right, and if μ is negative, it will be shifted to the left.

used this parameter comparing among different populations to test null hypothesis

parametric tests



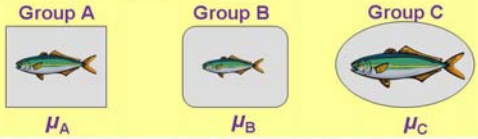
- *dispersion parameter* (σ^2)

or called a scale parameter, since its value determines the "scale" or statistical dispersion of the probability distribution. If σ^2 is large, then the distribution will be more spread out; if σ^2 is small then it will be more concentrated.

used its ratio for calculation of *F-value* in statistical test : Analysis of Variance (ANOVA)

Analysis of Variance (ANOVA)

A test (null hypothesis : H_0) of the statistical significance of the differences among the mean scores of more than two groups (or populations)



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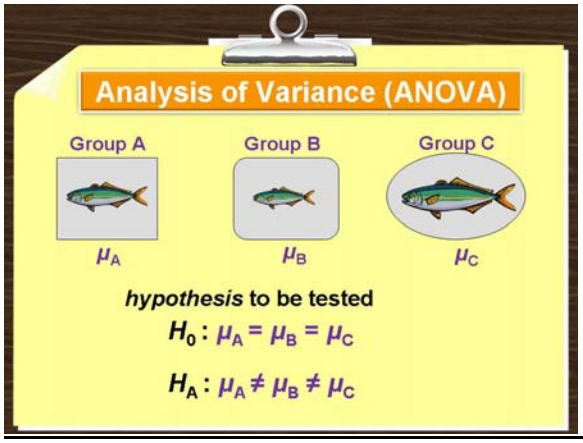
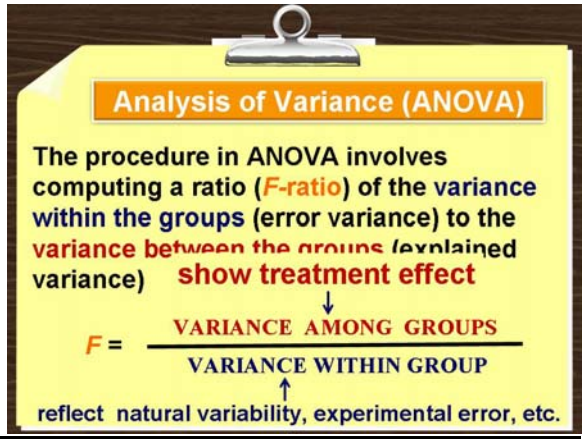
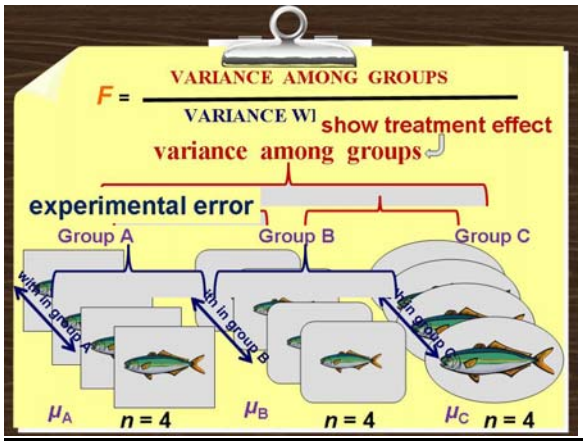
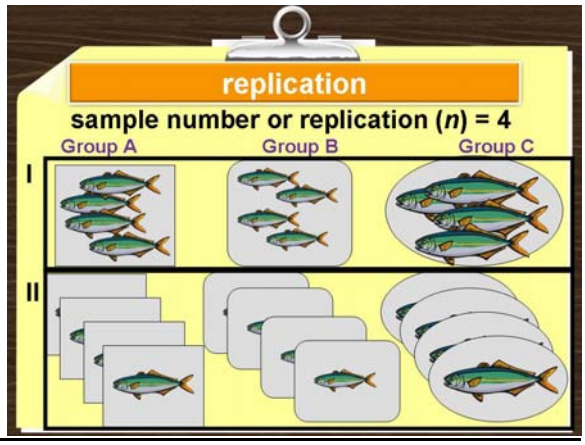
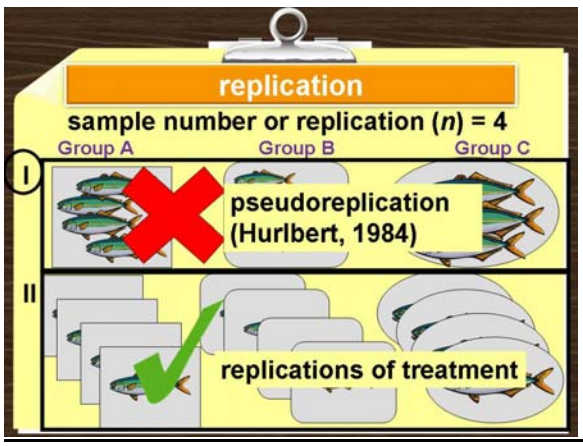
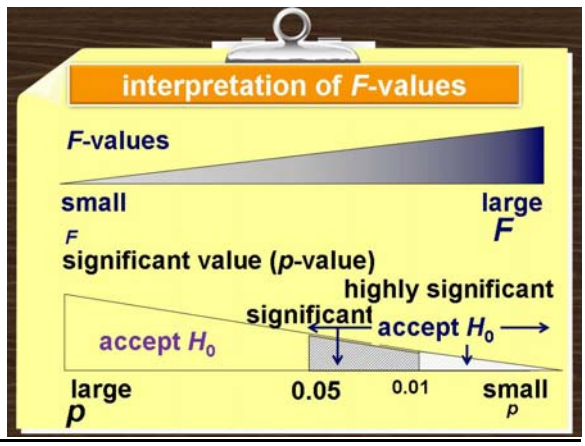

Analysis of Variance (ANOVA)
Group A
Group B
Group C
μ_A
μ_B
μ_C
hypothesis to be tested
$H_0: \mu_A = \mu_B = \mu_C$
$H_A: \mu_A \neq \mu_B \neq \mu_C$

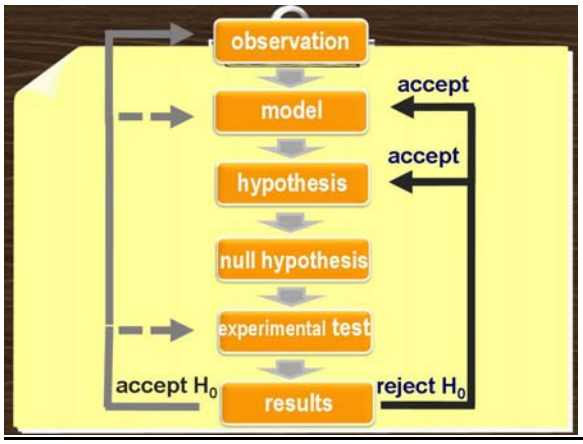

Analysis of Variance (ANOVA)
The procedure in ANOVA involves computing a ratio (*F*-ratio) of the variance within the groups (error variance) to the variance between the groups (explained variance)
show treatment effect
$F =$ VARIANCE AMONG GROUPS / VARIANCE WITHIN GROUP
reflect natural variability, experimental error, etc.

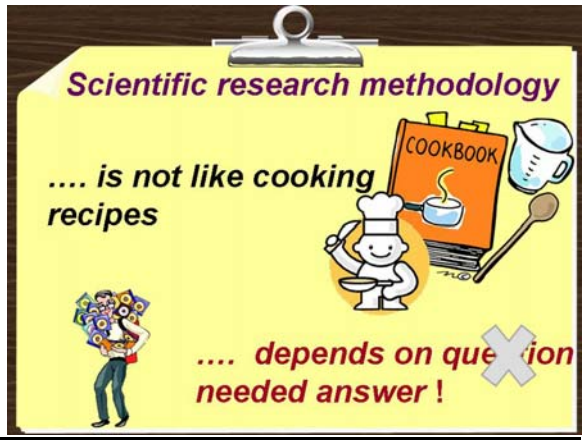

$F =$ VARIANCE AMONG GROUPS / VARIANCE WI
show treatment effect
variance among groups
experimental error
Group A
Group B
Group C
μ_A $n = 4$
μ_B $n = 4$
μ_C $n = 4$


replication
sample number or replication (*n*) = 4
Group A
Group B
Group C
I
II


replication
sample number or replication (*n*) = 4
Group A
Group B
Group C
I
II
pseudoreplication (Hurlbert, 1984)
replications of treatment


interpretation of *F*-values
F-values
small
large
F
significant value (*p*-value)
highly significant
significant
accept H_0
accept H_0
large
p
0.05
0.01
small
p


observation
model
hypothesis
null hypothesis
experimental test
results
accept
accept
accept H_0
reject H_0


Scientific research methodology
.... is not like cooking recipes
COOKBOOK
.... depends on question needed answer !

biological diversity

the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems.

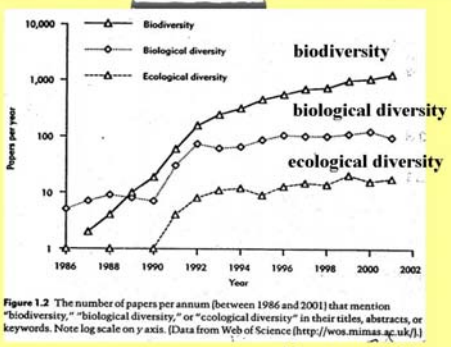
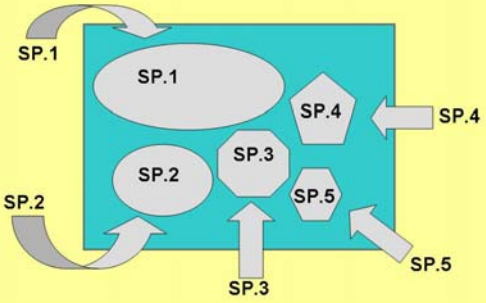


Figure 1.2 The number of papers per annum (between 1986 and 2001) that mention "biodiversity," "biological diversity," or "ecological diversity" in their titles, abstracts, or keywords. Note log scale on *y* axis. (Data from Web of Science (http://wos.mimas.ac.uk/).)

Magurran, 2004

SPATIAL DISTRIBUTION



Species diversity

- Species richness (number of species)

any value depending on occurrence species found in the samples

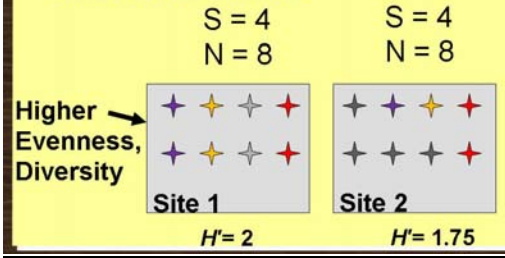
1. species numbers depending on :
   - individual numbers
   - sample size and sample number
2. Margalef's index

$$D_{Mg} = \frac{(S-1)}{\log N}$$

S = total numbers of species
N = total individual numbers

evenness increases diversity

- increasing evenness → greater diversity
  - –true for all indices



Species diversity index

Shannon diversity index (H')

$$p_i = \frac{n_i}{N}$$

k = species numbers in each sample

$$H' = -\sum_{i=1}^{k} p_i (\log p_i)$$

the relative abundance of each species

species richness

unit of index
$\log_2$ " bit "
ln " nat"
$\log_{10}$ " decit"

Pielou's evenness value

$$J' = \frac{H'(observed)}{H'_{max}}$$

H'_{max} the index is maximized when each species is present in equal numbers

$$H'_{max} = \ln S$$

	St.1	St.2	St.3	St.4	St.5	St.6
Sp.1	20	40	40	100	120	160
Sp.2	20	32	40	65	45	34
Sp.3	20	25	40	28	24	4
Sp.4	20	20	40	1	4	1
Sp.5	20	18	40	1	2	1
Sp.6	20	16	0	1	1	0
Sp.7	20	14	0	1	1	0
Sp.8	20	13	0	1	1	0
Sp.9	20	12	0	1	1	0
Sp.10	20	10	0	1	1	0
Sp.11	0	0	0	0	1	0
Sp.12	0	0	0	0	1	0
Sp.13	0	0	0	0	1	0
Sp.14	0	0	0	0	1	0
Sp.15	0	0	0	0	1	0
Sp.16	0	0	0	0	1	0
Sp.17	0	0	0	0	1	0
Sp.18	0	0	0	0	1	0
Sp.19	0	0	0	0	1	0
Sp.20	0	0	0	0	1	0
total abun.	200	200	200	200	200	200

	Highest value	High value	Medium value	Medium value	Medium value	Low value
	St.1	St.2	St.3	St.4	St.5	St.6
H'(loge)	2.30	2.21	1.61	1.17	1.15	0.61
no of species	M 10	M 10	L 5	M 10	H 20	L 5
Evenness (J')	H 1.00	H 0.96	H 1.00	L 0.51	L 0.39	L 0.38
dominant sp.	no	no	no	yes	yes	yes

Results of analysis of species diversity indices

Species composition & abundance of macrobenthos in Phuket seagrass bed

Annex 7


conclusion
Good observation
Good reading
Good study design
Good data quality
Good finding
Thank you for your attention
Sawasdee ka

Annex 8: General Procedures for Sampling, Identification and Collection Management of Deep-Sea Fishes

By Dr. Yoshinobu Konishi

Annex 8

General Procedure for Identification and Collection Management of Deep-sea Fishes

Content

- Research on the fisheries resources exploration of deep-sea bottom fishes and impacts of the fishing to the ecosystem
- General procedure for identification and collection management of deep-sea fishes

Yoshinobu KONISHI

(Retired from the Seikai National Fisheries Research Institute in Nagasaki, Japan in 2009)

Topographic recognition of deep-sea demersal fisheries in the Southeast Asian region

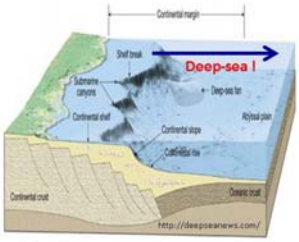


Fig. by Matsushita at the deep-sea meeting in 2009

- **100 – 200 m depth** (shelf break area) due to low utilization for demersal fisheries
- ◎ **200 m depth –** (international standard on deep sea area) important demersal fishes inhabit in continental slope area (200 - 500 m depth)

* Note: 1) fish fauna is different between the shelf break and continental slope areas, 2) the fauna in the shelf break area is nearly same as that in the coastal area less than 100 m depth.

Deep sea ranging from 200 – 1000 m depth and past surveys on deep-sea demersal fisheries resources exploration in the region

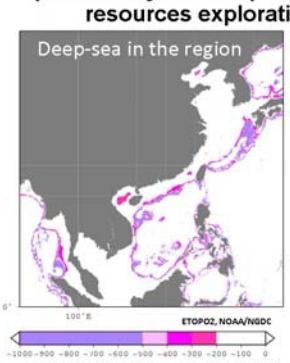


Fig. by Matsushita at the deep-sea meeting in 2009

Main surveys after 1980

- Brunei Darussalam (SCS)
- Indonesia (W-N of Sumatra Isl., S of Java Isl.)
- Malaysia (off Sarawak & Sabah)
- Myanmar (Andaman Sea)
- Philippines (Luzon, Mindro, Palawan, Lingayen Gulf etc)
- Thailand (Andaman Sea)
- Vietnam (off the central Vietnam)
- SEAFDEC-TD (Brunei waters, Sabah & Sarawak waters, Andaman Sea)

Some important demersal fishes for deep-sea fisheries in the region

Alfonsino (***Beryx splendens***)
Photo by Satria at the deep-sea meeting in 2010

Seapearch (***Malakichthys wakiyae***)

Deep-water snapper (***Etelis radiosus***)
Photo by Satria at the deep-sea meeting in 2010

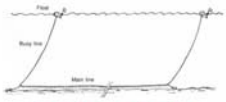
Rattail (***Caelorichus*** sp.)

Mirror dory (***Zenopsis nebulosa***)
Photo from *Fishbase*

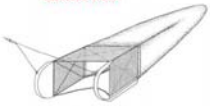
Roughy (***Hoplostethus crassispinus*?**)
Photo by Satria at the deep-sea meeting in 2010

Some fishing gears for deep-sea demersal fishes

Bottom trawl



Bottom horizontal longline



Beam trawl

Trap

Note: gear(s) to be used is depending on the sea bed topography and substratum, and has fish-size and species selectivity.

Expected impacts of deep-sea demersal fisheries to the bottom ecosystem

- Destruction of the vulnerable ecosystem (corals, sponge, starfish and sea urchin)
- Reduction of stock size of targeted fishes and then collapse of the resources by the high fishing pressure
  Deep-sea fishes: slow growth, long life span, late mature age
- Simplicity of the biodiversity in marine organisms such as fishes and crustacea, that enable to be captured by fisheries
- Structural change of the ecosystem

Toward to the fisheries resources exploration of deep-sea demersal fishes and their management in the Southeast Asian region [1/2]

Early-step subjects

1. Identification of possible fishing grounds by topographic and substratum surveys and fish echo surveys with the echo sounder (mainly at 200-500 m depth area)
2. Identification of commercially important fishes and crastacea by fishing surveys with various gears, and acquisition of their spatial distributions and abundances (including fish larvae and juveniles)
3. Mapping of possible fishing grounds for each fishing gear and important species
4. Identification of the aggregated vulnerable-ecosystem organisms by visual surveys with a drop camera system and ROV to set the fishing preserve for conservation of the ecosystems

Toward to the fisheries resources exploration of deep-sea demersal fishes and their management in the Southeast Asian region [2/2]

Middle- and long-term subjects

5. Growth, recruitment, reproduction (spawning season) of important fish species as the basic information for the fisheries management
6. Fish food web, and physical and biological environment for understanding the structure and interaction of the ecosystem
7. Monitoring of the fish and crastacea diversities and abundances of the targeted species for the precautionary fisheries management
8. Development and improvement of fishing gears for reduction of the incidental catch of untargeted fishes and impacts to the vulnerable ecosystems
9. Life-history study of vulnerable-ecosystem organisms

Procedure of fish collection

1 Sampling of deep-sea fishes
- on-board sampling with sampling gears
- fish-market sampling

2 Handling of fish specimens
- freezing
- cold storage with ice
- preservation in 10% formalin solution

3 Identification
- photography
- muscle sampling for DNA analysis

4 Collection management
- registration of specimens in database
- storage of registered specimens in the dark and cool space, and the tissues in refrigerater

◎ *Request of identification for unknown specimens*

2. Handling of fish specimens

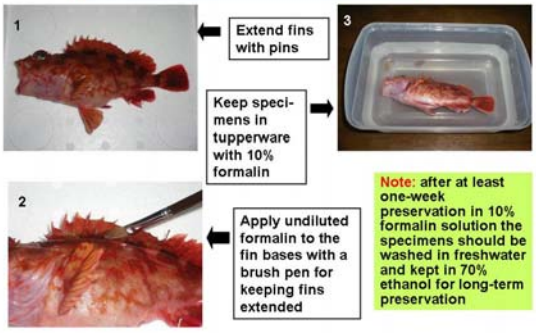
Freezing (on board)
- Specimens are kept frozen until identification in laboratory
- To avoid drying the specimens, each of them is better to be kept into a plastic bag or be covered with wrap

Cold storage with ice (on board, at fish market)
- Specimens are kept in a cooler with ice until identification in laboratory

Preservation in 10% formalin solution (on board, at fish market)
- Under no freezer or limit of capacity of the freezer at specimen sampling/handling, the specimens should be preserved in 10% formalin solution
- Muscle tissues in right-side body of specimen to be registered in database should be sampled before preservation with formalin

Note: specimens which have characteristic body color and/or pigment patterns on the fin membranes are better to be taken photo prior to the handling above

Preservation of specimens in 10% formalin solution



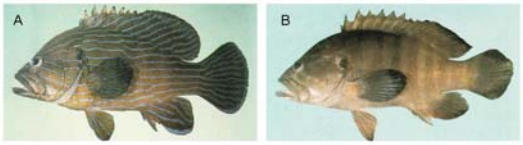
Note: after at least one-week preservation in 10% formalin solution the specimens should be washed in freshwater and kept in 70% ethanol for long-term preservation

Example of characteristic body color and pigment on body and fins

A part of key to Indo-Pacific species of *Cephalopholis* (from FAO species catalogue, vol. 16)

7a. Pectoral fins short, their length contained 1.5 to 1.8 times in head length; color generally brown or yellowish brown, with dark blue lines on head, body and fins (Fig. A) ·····*C. formosa*

7b. Pectoral fins 1.3 to 1.6 in head length; body brown, usually with 7 or 8 dark bars; no blue lines on head or body; fins dark brown, with a pale blue line at corners of caudal (Fig. B) ·····*C. boenak*



3. Identification (laboratory work)

Identification
- Defrosting of frozen specimens prior to identification (sometimes from one-day before)
- Identification of specimens with references

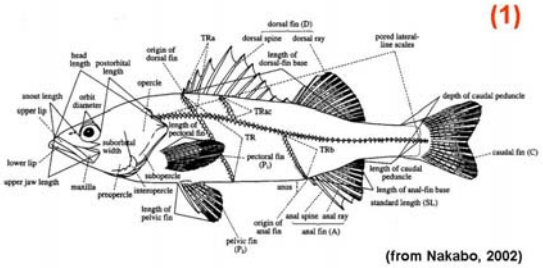
Photography
- Taking pictures of important specimens scientifically

Tissues sampling for DNA analysis
- Sampling of muscle in the right-side body for specimens to be registered in database

* DNA analysis is useful for verification of the original identification and larval fish identification

Preservation of specimens
- Preservation of fresh specimens in 10% formalin solution for collection (the specimens should be transferred into 70% ethanol 1 week to 1 month later)

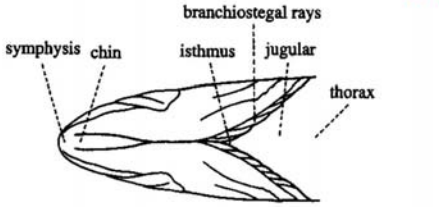
External characters of bony fish and methods of measuring (1)



(from Nakabo, 2002)

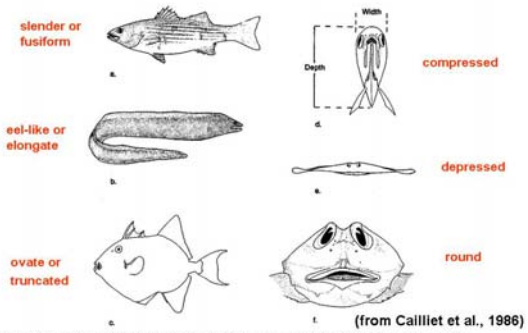
Nakabo, T. (ed.) 2002: Fishes of Japan with pictorial keys to the species, English edition. Tokai University Press, Tokyo, 1749 pp.

External characters of bony fish and methods of measuring (2)



(from Nakabo, 2002)

Body shapes of bony fish

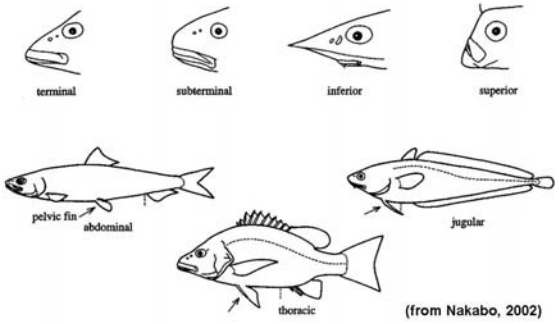


(from Cailliet et al., 1986)

Cailliet, G. M., Love, M. S. and Ebeling A. W. 1986: Fishes. Field and laboratory manual on their structure, identification, and natural history. Wadsworth Publishing Company, California, 194 pp.

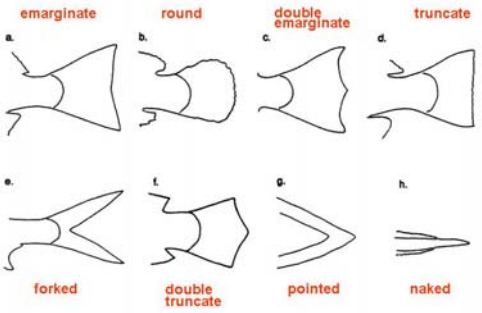
Annex 8

Position of mouth and pelvic fins



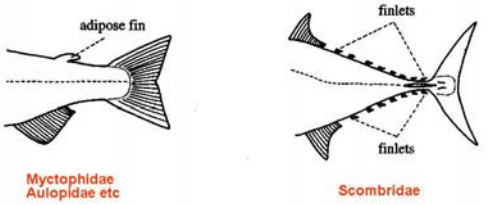
(from Nakabo, 2002)

Caudal fin forms



(modified Cailliet et al., 1986)

Adipose fin and finlets



Myctophidae
Aulopidae etc

Scombridae

Adipose fin is membranous.
Finlet has fin element (like ray).

(from Nakabo, 2002)

Methods of measurements

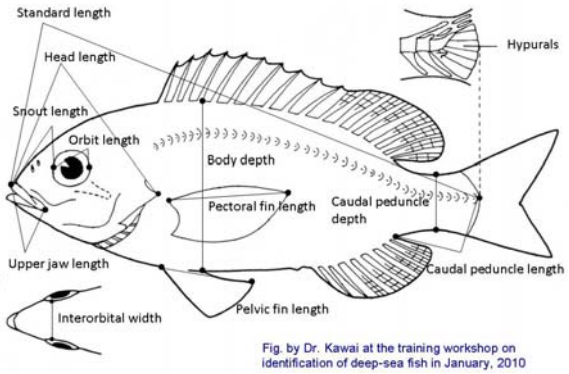


Fig. by Dr. Kawai at the training workshop on identification of deep-sea fish in January, 2010

Methods of counts

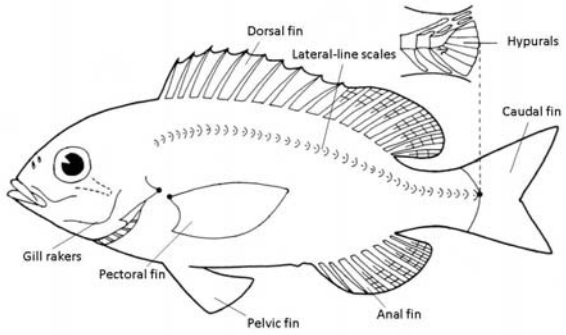
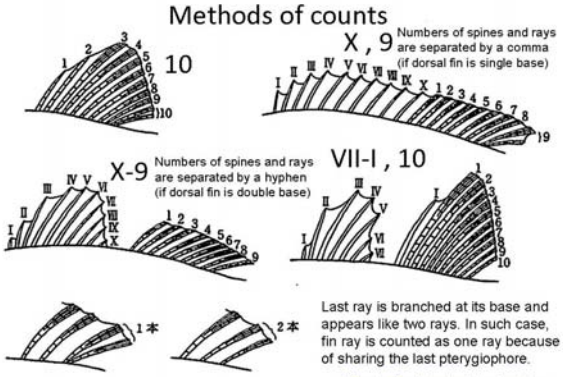


Fig. by Dr. Kawai at the training workshop on identification of deep-sea fish in January, 2010

Methods of counts

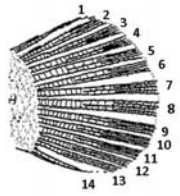


Last ray is branched at its base and appears like two rays. In such case, fin ray is counted as one ray because of sharing the last pterygiophore.

Spines: Roman numerals Soft rays: Arabic numerals

Fig. by Dr. Kawai at the training workshop on identification of deep-sea fish in January, 2010

Principal caudal fin counts



Branched rays +Two unbranched rays

12+2=14

Fig. by Dr. Kawai at the training workshop on identification of deep-sea fish in January, 2010

Some useful references for identification of fishes in the Southeast Asian region

http://www.fao.org/docrep/009/x2400e/x2400e00.HTM

FishBase:
http://www.fishbase.org/
Catalog of Fishes:
http://research.calacademy.org/ichthyology

Nakabo, T. (ed.) 2002: Fishes of Japan with pictorial keys to the species (English edition). Tokai University Press, Tokyo, 1749pp.

Heemstra, P. C. and J. E. Randall. 1993: Groupers of the world (family Serranidae, subfamily Epinephelinae). FAO Fisheries Synopsis, no. 125, vol.16, 382pp.

Annex 8

Photography and tissues sampling

Photos:
Pristigenys niphonia (upper)
Callanthias japonicus (lower)

Tissues samples in 90% ethanol

- Cut a small piece of muscle in the right-side body (two pieces/specimen)
- Put the piece and a label into a vial with 90% ethanol
- Keep a tupperware with vials in a refrigerator as tissues collection

4. Collection management

- registration of specimens into database
- storage of the registered specimens in the dark and cool space, and the tissues samples in refrigerator

Input items of database
- catalogue (bottle) number
- genus name
- species name
- no. of individuals
- min. body length (mm)
- max. body length (mm)
- TL/FL/SL
- body weight (g)
- family name
- order name
- sampling position/place
- sampling date
- sampling gear/method
- sampling person
- identification person
- vial no. of tissues

Preserved specimen and a water-proof label (catalogue no., species, sampling position, sampling date, family)

Storage shelf

Package of specimens for request of identification

Fig. 1

Fig. 2

1. Roll a specimen by wet gauze with the preserved solution (Fig. 1)
2. Put the specimen into a reinforced plastic bag (Fig. 2)
3. Seal the opening portion of the plastic bag by impulse sealer
4. Put the plastic bag with the specimen into another plastic bag and seal the outside plastic bag

Package of specimens for request of identification

Fig. 3

Fig. 4

5. Roll the double plastic bag with the specimen by plastic sheet with air cells
6. Put the specimen rolled by plastic sheet into a box (Fig. 3)
7. Cover the box with hard paper and stick a sticker of "Scientific specimen of fish preserved" (Fig. 4)
8. Send the parcel (or EMS) with the specimen and its data to an expert

On-board works in the deep-sea demersal fish survey

For each sampling station and gear

1. Sorting and identification
2. Measurements of number and weight of captured fishes for each species including the unknown specimens
3. Body length punching of at least 50-100 specimens for the selected species
4. Taking photos for the selected species, in particular fishes with the characteristic body color and pigment patterns, and the unknown specimens
5. Freezing/icing or preservation by 10% sea water-formalin for 50-100 specimens of the selected fishes for detailed and precise examination in the laboratory (including the unknown specimens)

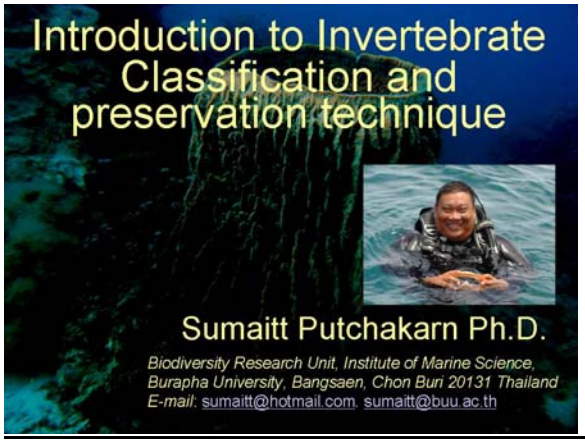
Until the end of the survey cruise

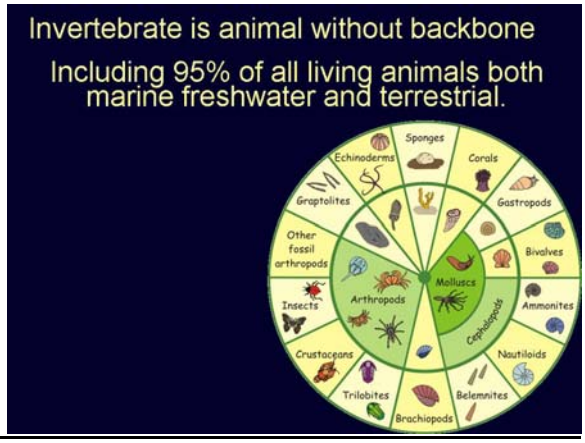
6. Making the catch data table with gear operation data and the size composition figures

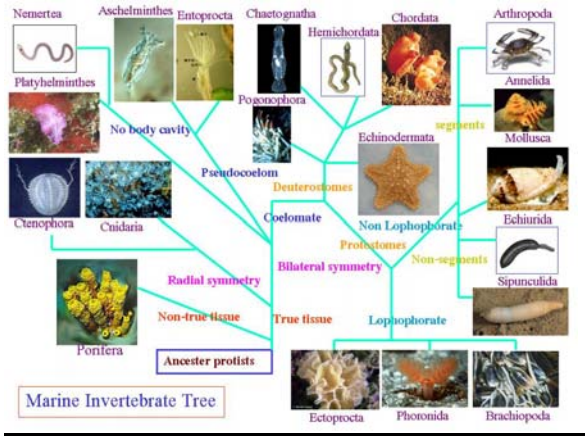
Dishes with unfamiliar deep-sea fishes at the taste party

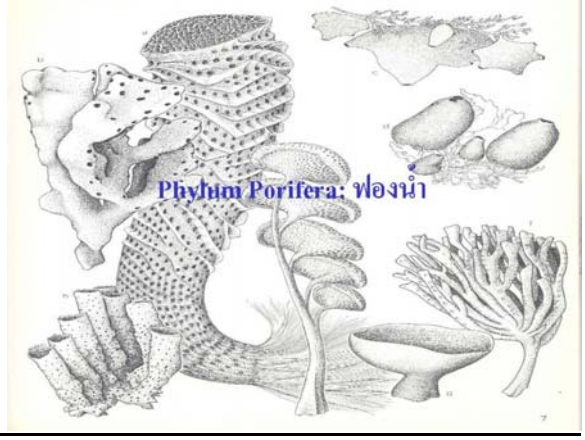
Annex 9: Introduction to Invertebrate Classification and Preservation Technique

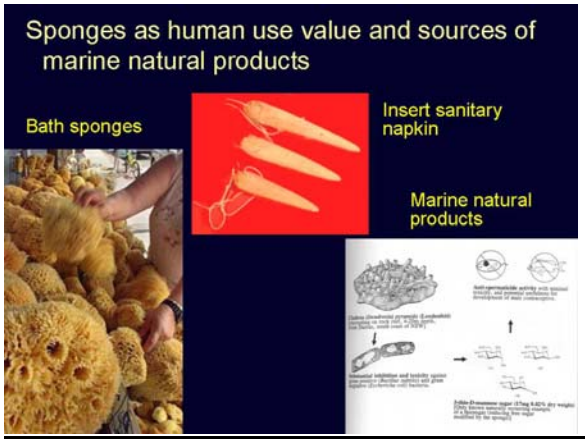
By Dr. Sumaitt Putchakarn

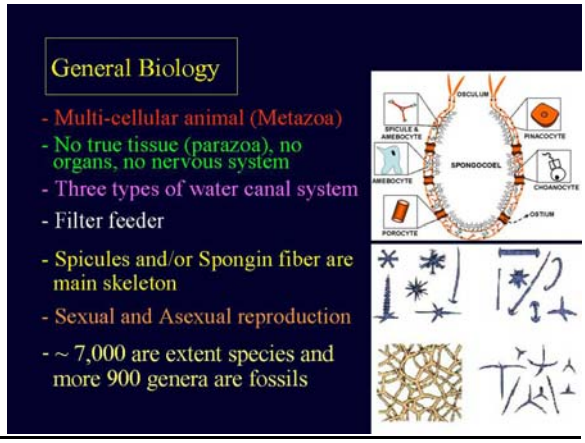

Introduction to Invertebrate Classification and preservation technique
Sumaitt Putchakarn Ph.D.
Biodiversity Research Unit, Institute of Marine Science, Burapha University, Bangsaen, Chon Buri 20131 Thailand
E-mail: sumaitt@hotmail.com, sumaitt@buu.ac.th

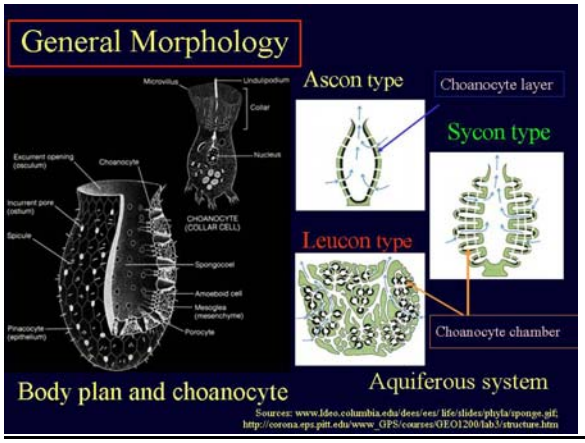

Invertebrate is animal without backbone
Including 95% of all living animals both marine freshwater and terrestrial.
Sponges
Corals
Gastropods
Bivalves
Ammonites
Nautiloids
Belemnites
Brachiopods
Trilobites
Crustaceans
Insects
Other fossil arthropods
Graptolites
Echinoderms
Molluscs
Arthropods
Cephalopods

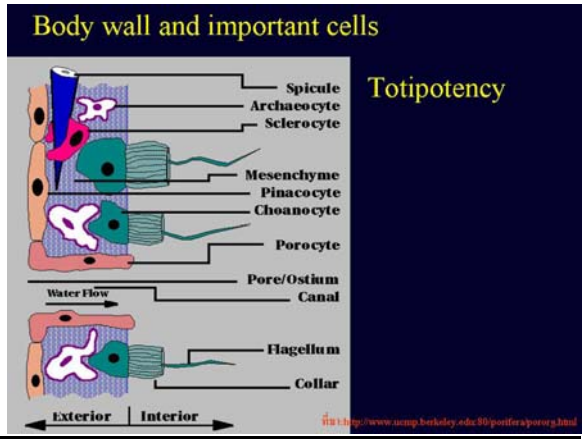

Nemertea
Aschelminthes
Entoprocta
Chaetognatha
Hemichordata
Chordata
Arthropoda
Platyhelminthes
Annelida
Pogonophora
No body cavity
segments
Mollusca
Echinodermata
Pseudocoelom
Deuterostomes
Ctenophora
Cnidaria
Coelomate
Non Lophophorate
Echiurida
Protostomes
Non-segments
Radial symmetry
Bilateral symmetry
Sipunculida
Non-true tissue
True tissue
Lophophorate
Porifera
Ancester protists
Marine Invertebrate Tree
Ectoprocta
Phoronida
Brachiopoda


Phylum Porifera: ฟองน้ำ

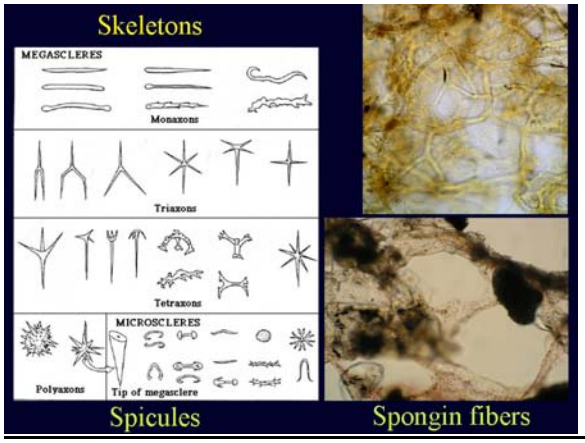

Sponges as human use value and sources of marine natural products
Bath sponges
Insert sanitary napkin
Marine natural products

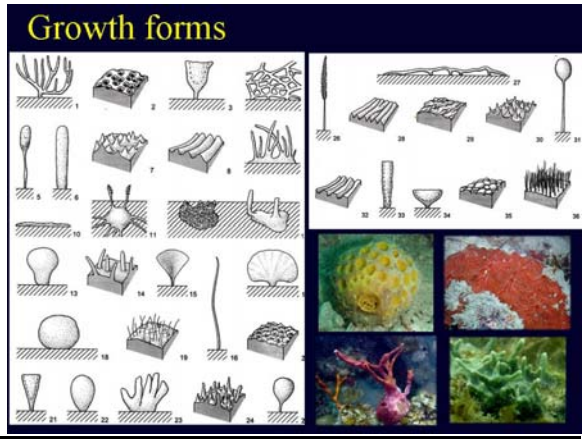

General Biology
- Multi-cellular animal (Metazoa)
- No true tissue (parazoa), no organs, no nervous system
- Three types of water canal system
- Filter feeder
- Spicules and/or Spongin fiber are main skeleton
- Sexual and Asexual reproduction
- ~ 7,000 are extent species and more 900 genera are fossils
OSCULUM
SPICULE & AMEBOCYTE
PINACOCYTE
SPONGOCOEL
AMEBOCYTE
CHOANOCYTE
POROCYTE
OSTIUM

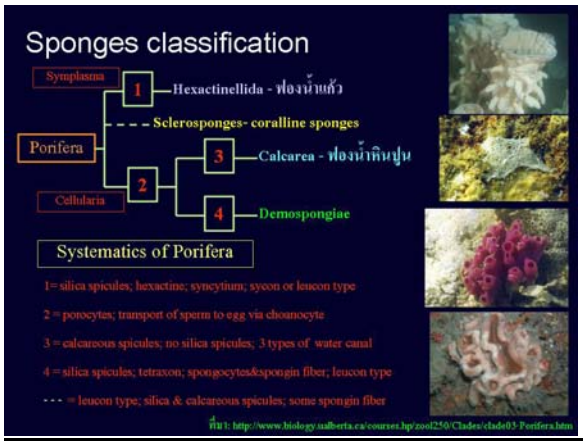

General Morphology
Ascon type
Choanocyte layer
Sycon type
Leucon type
Choanocyte chamber
Body plan and choanocyte
Aquiferous system
Sources: www.ldeo.columbia.edu/dees/ees/ life/slides/phyla/sponge.gif; http://corona.eps.pitt.edu/www_GPS/courses/GEO1200/lab3/structure.htm

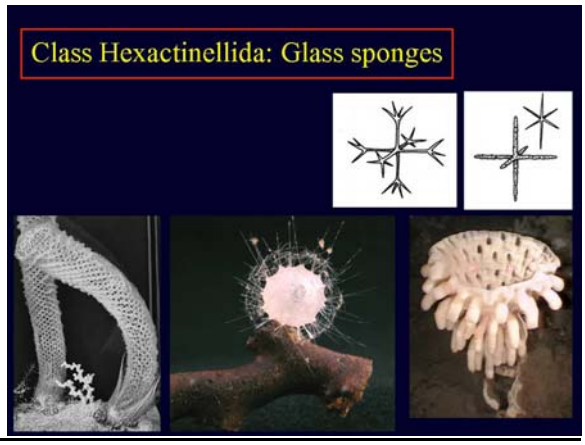

Body wall and important cells
Spicule
Archaeocyte
Sclerocyte
Mesenchyme
Pinacocyte
Choanocyte
Porocyte
Pore/Ostium
Canal
Water Flow
Flagellum
Collar
Exterior
Interior
Totipotency

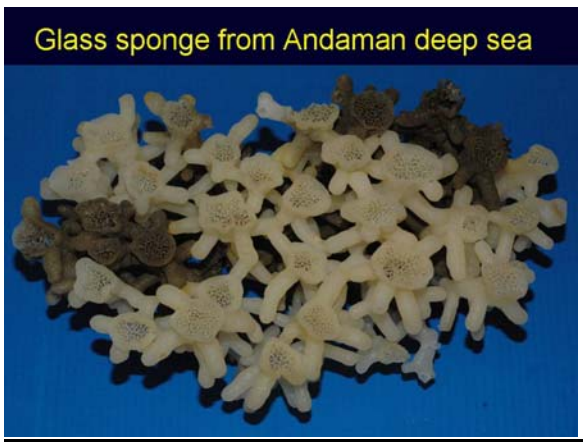
Annex 9

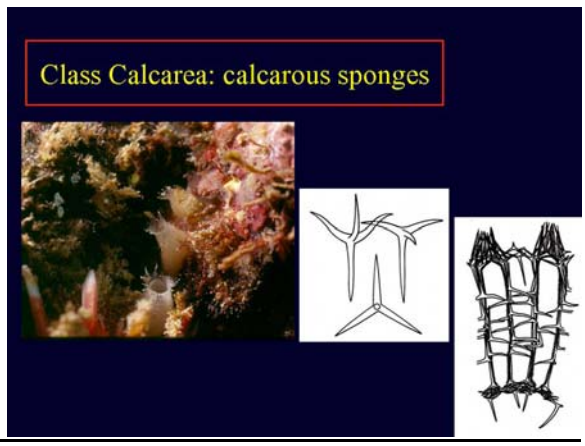

Skeletons
MEGASCLERES
Monaxons
Triaxons
Tetraxons
MICROSCLERES
Polyaxons
Tip of megasclere
Spicules
Spongin fibers

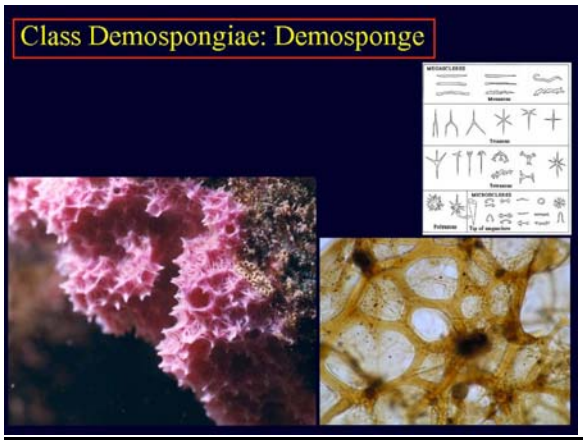

Growth forms


Sponges classification
Symplasma
1
Hexactinellida - ฟองน้ำแก้ว
Sclerosponges- coralline sponges
Porifera
3
Calcarea - ฟองน้ำหินปูน
2
Cellularia
4
Demospongiae
Systematics of Porifera
1= silica spicules; hexactine; syncytium; sycon or leucon type
2 = porocytes; transport of sperm to egg via choanocyte
3 = calcareous spicules; no silica spicules; 3 types of water canal
4 = silica spicules; tetraxon; spongocytes&spongin fiber; leucon type
- - - = leucon type; silica & calcareous spicules; some spongin fiber
ที่มา: http://www.biology.ualberta.ca/courses.hp/zool250/Clades/clade03-Porifera.htm


Class Hexactinellida: Glass sponges

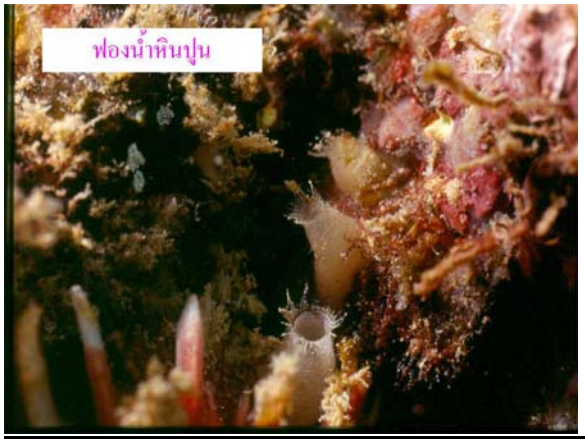

Glass sponge from Andaman deep sea


Class Calcarea: calcarous sponges


Class Demospongiae: Demosponge


Class Demospongiae: Coralline sponge

Annex 9


ฟองน้ำหินปูน

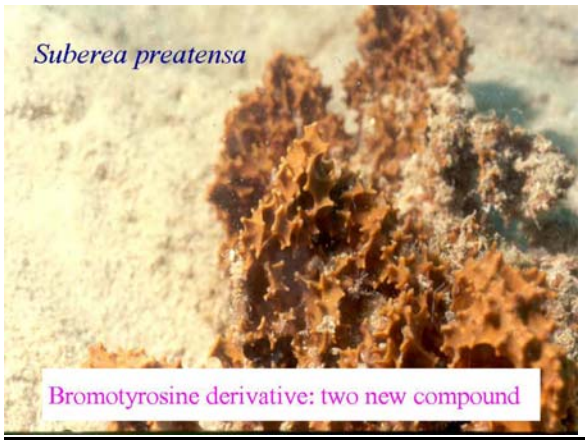

ฟองน้ำหินปูน


ฟองน้ำหินปูน


ฟองน้ำเคลือบหินสีน้ำตาล


ฟองน้ำลูกกอล์ฟ


Tetilla japonica
Tetrahydrofurylhydro-xypyran-2-one: two new compounds


Suberea preatensa
Bromotyrosine derivative: two new compound


ฟองน้ำเปลี่ยนสี, Pseudoceratina sp.

Annex 9


ฟองน้ำแคลือบสีม่วง

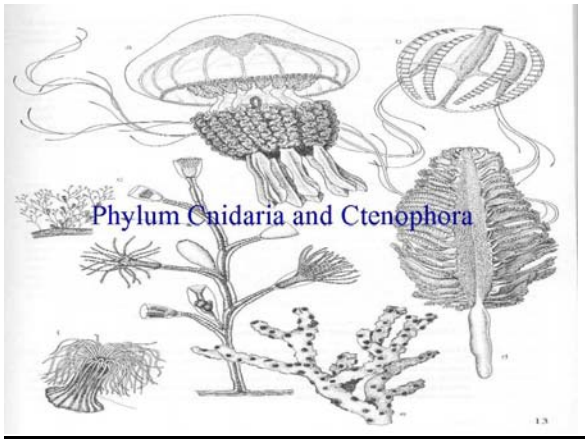
Annex 9


ฟองน้ำสีชมพู


ฟองน้ำสีน้ำเงิน


ฟองน้ำเส้นหมี่


ฟองน้ำหูช้าง


Phylum Cnidaria and Ctenophora


Class Hydrozoa
ไฮดรอยด์


ขนนกทะเล


แว่นตาพระอินทร์

Annex 9


ปะการังไฟแผ่น


ปะการังไฟกิ่ง


Class Scyphozoa
แมงกะพรุนหนัง


แมงกะพรุนไฟ

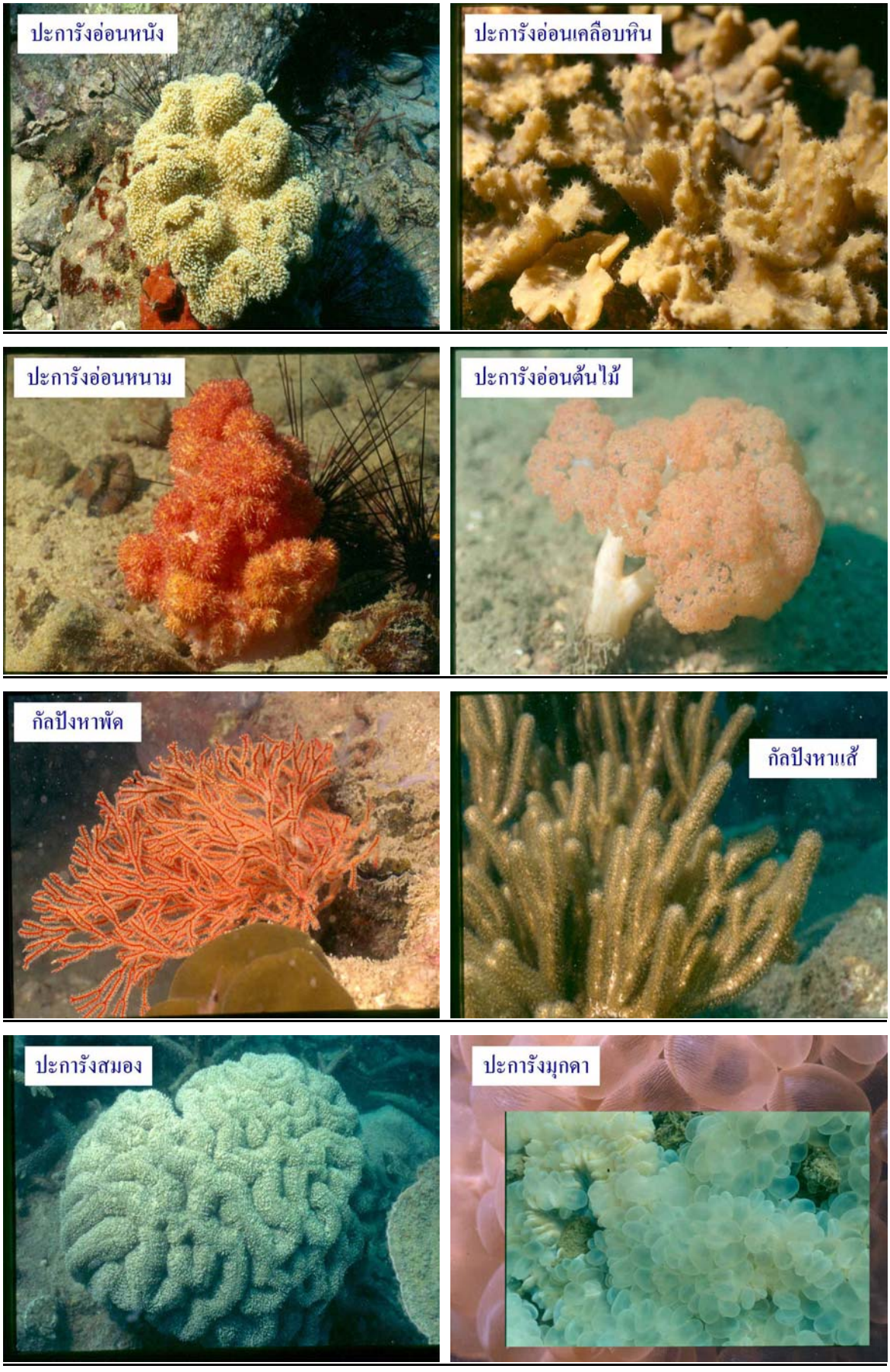

Class Anthozoa
ปะการังสีน้ำเงิน


ปากกาทะเล


ปะการังอ่อน


ปะการังอ่อนนิ้วมือ

Annex 9


ปะการังอ่อนหนัง
ปะการังอ่อนเคลือบหิน
ปะการังอ่อนหนาม
ปะการังอ่อนต้นไม้
กัลปังหาพัด
กัลปังหาแส้
ปะการังสมอง
ปะการังมุกดา

Annex 9


ปะการังโต๊ะ

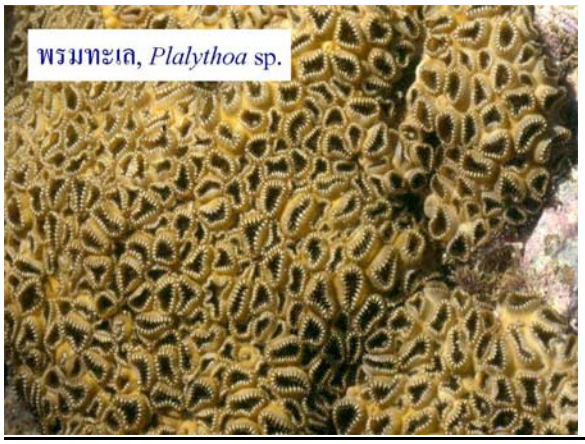

ปะการังเขากวาง


ปะการังเห็ด


ปะการังถ้วยส้ม

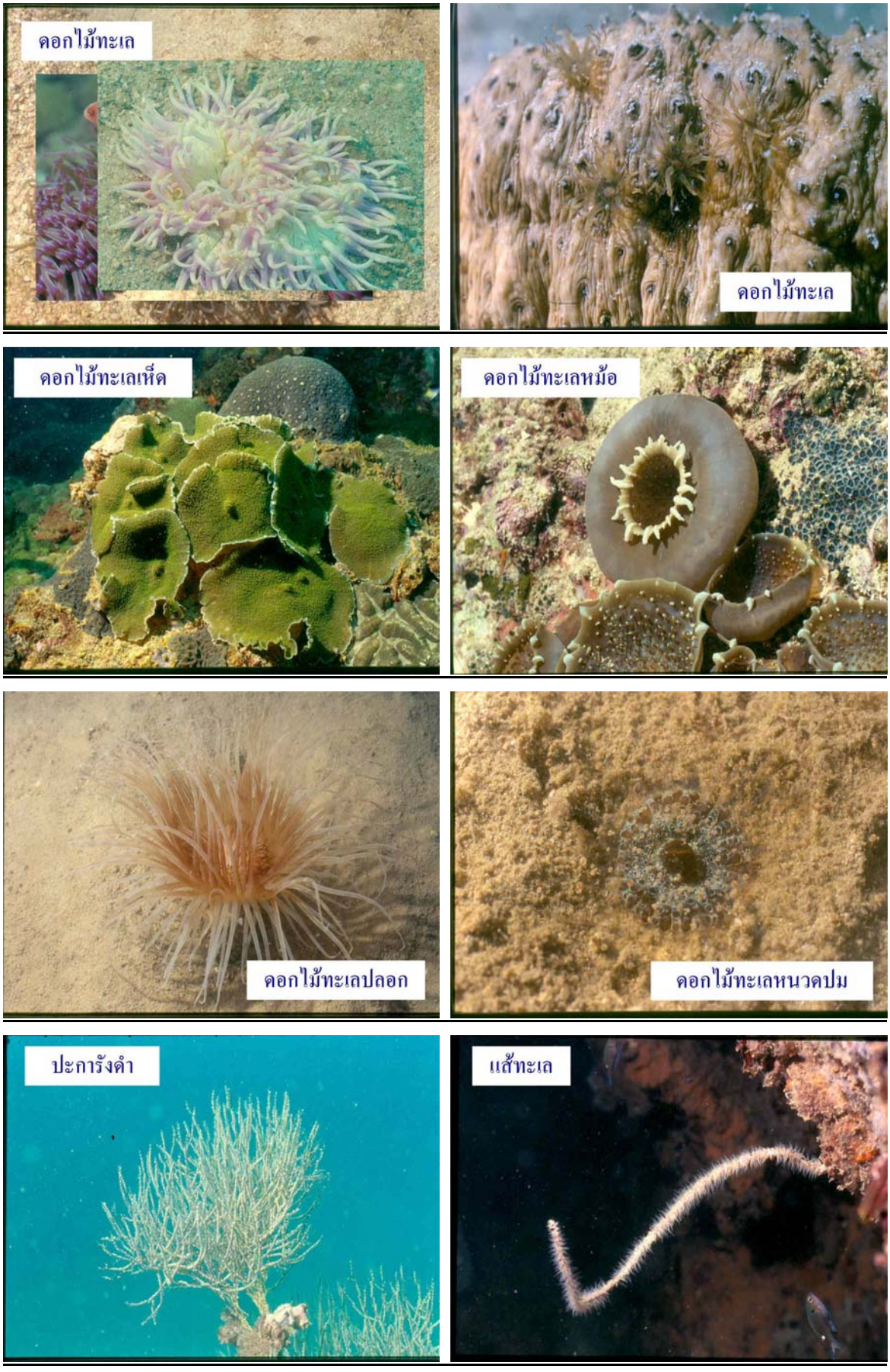

ปะการังเม็ดกระดุม


พรมทะเล, Zoanthus sp.
New ecdysteroid compound: Zoanthusterone

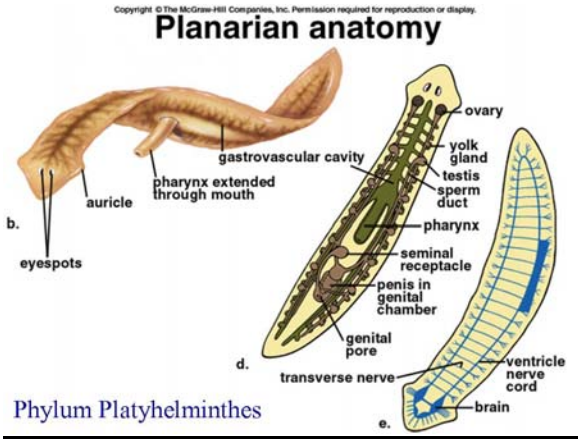

พรมทะเล, Plalythoa sp.


ดอกดิน

Annex 9


ดอกไม้ทะเล
ดอกไม้ทะเล
ดอกไม้ทะเลเห็ด
ดอกไม้ทะเลหม้อ
ดอกไม้ทะเลปลอก
ดอกไม้ทะเลหนวดปม
ปะการังดำ
แส้ทะเล

Annex 9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Planarian anatomy
gastrovascular cavity
pharynx extended through mouth
auricle
eyespots
b.
ovary
yolk gland
testis
sperm duct
pharynx
seminal receptacle
penis in genital chamber
genital pore
d.
transverse nerve
ventricle
nerve cord
brain
e.
Phylum Platyhelminthes


Phylum Platyhelminthes
หนอนตัวแบน


หนอนตัวแบน


Phylum Nemertea
proboscis opening
proboscis
retractor muscle
หนอนริบบิ้นม้าลาย

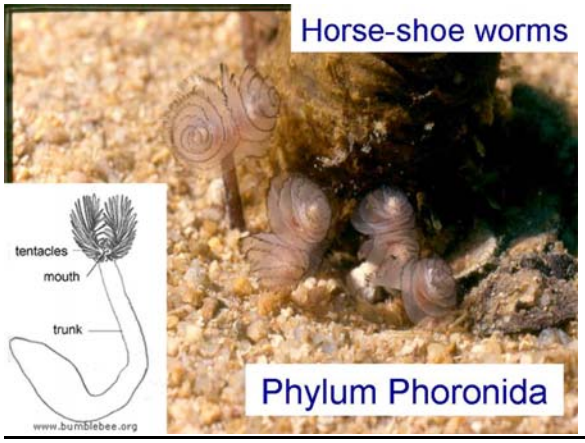

mouth
anus
stomach
stalk
Phylum Entoprocta
Goblet worms


Phylum Ectoprocta
Bryozoa

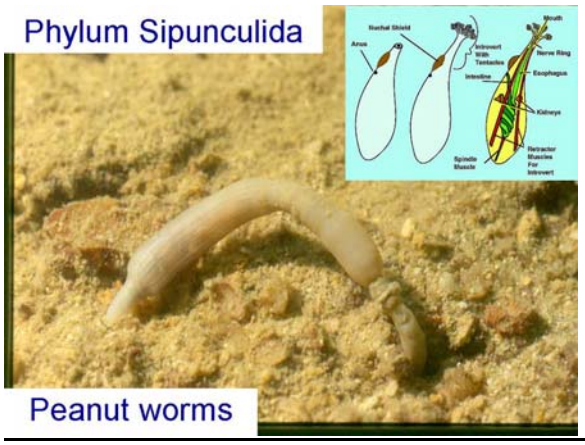

ไบรโอซัวรู่ม


ไบรโอซัวเส้นหมี่

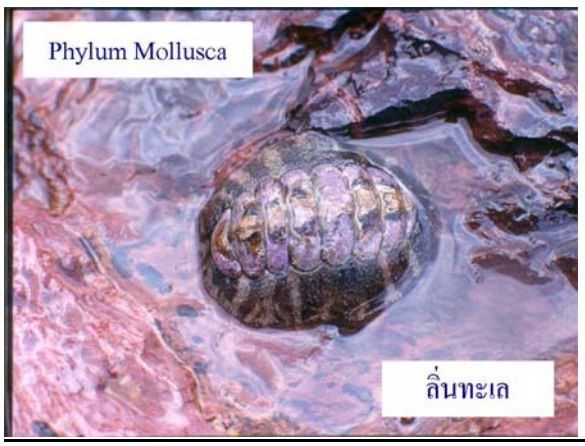
Annex 9


Horse-shoe worms
tentacles
mouth
trunk
www.bumblebee.org
Phylum Phoronida


Phylum Brachiopoda
Lamp shell


Phylum Sipunculida
Peanut worms


Phylum Echiurida
หนอนช้อน


Phylum Mollusca
ลิ่นทะเล


หอยมือผี


หอยม่วง


หอยสังข์หน้ายักษ์

Annex 9


หอยเบี้ยปะการังอ่อน


กระต่ายทะเล


กระต่ายทะเล


ทากเปลือย


หอยมุกขนนกทะเล


หอยปะการัง


หอยมือเสือ


หอยมือแม่ว

Annex 9


หอยจาวมะพร้าว


หอยกระโดด


หอยจอบ


หมึกกระดอง


หมึกสาย


Phylum Annelida
แม่เพรียง


บุ้งทะเล


หนอนเกลี็ด

Annex 9


หนอนท่อ


หนอนท่อหินปูน


หนอนดอกไม้พู่ฉัตร


หนอนดอกไม้พายหางนกยูง


Phylum Arthropoda
แมงดาทะเลหางกลม

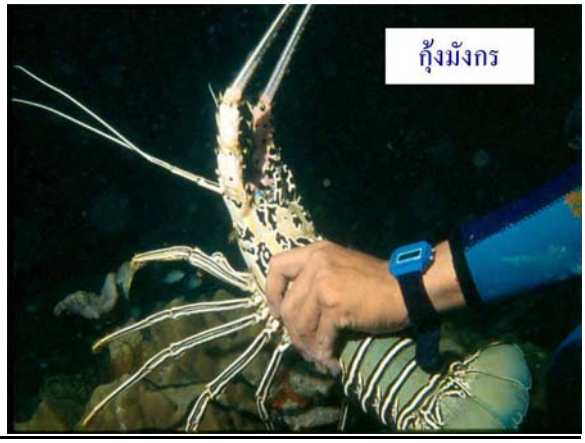

แม่หอบ


ปลาบู่กับกุ้งตาบอด


กุ้งแม่บ้านกับหอยจอบ

Annex 9


กุ้งแม่บ้านกับดอกไม้ทะเล


กุ้งมังกร

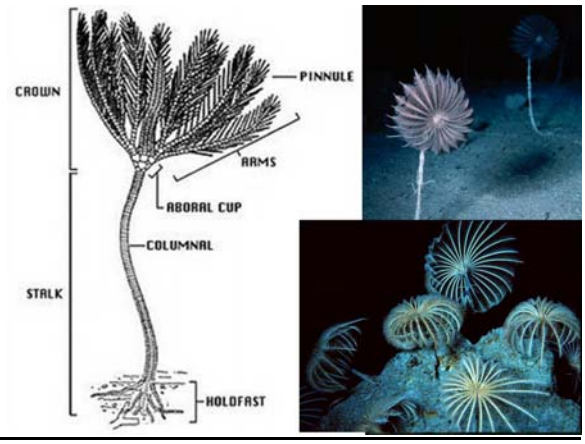

ปูเสฉวนยักษ์


จั๊กจั่นทะเล


ปูใบ้หลังเต่า


ปูม้าลายกับแม่แต่งตัว

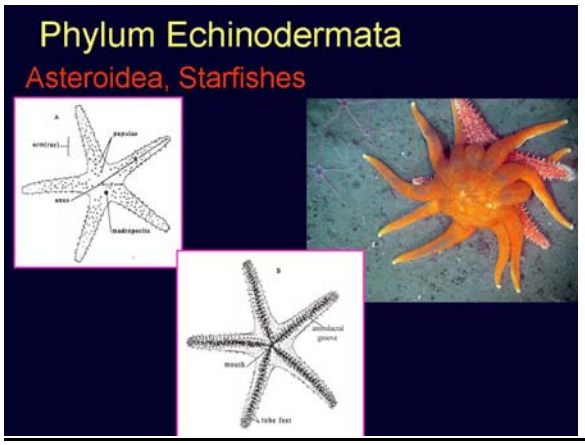

Phylum Echinodermata
Divided into 5 Clases:
Crinoidea
Sea lily, feather star


CROWN
PINNULE
ARMS
ABORAL CUP
COLUMNAL
STALK
HOLDFAST

Annex 9


ดาวขนนก


ความผันแปรสีของ Lamprometra palmata


Phylum Echinodermata
Asteroidea, Starfishes

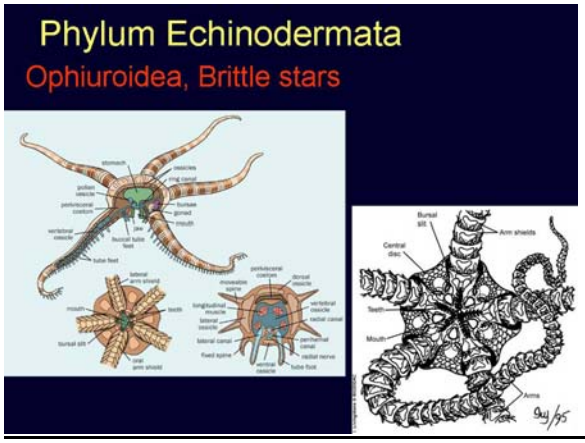

Phylum Echinodermata
Asteroidea, Starfishes


พฤติกรรมการกินอาหารของดาวแสงอาทิตย์
ดาวแสงอาทิตย์, Luidia maculata


ดาวหมอนปักเข็ม, Culcita novaeguineae Muller & Troschel, 1842


ดาวมงกุฎหนาม, Acanthaster planci


ดาวหนาม, Asteropsis carinifera


Phylum Echinodermata
Ophiuroidea, Brittle stars


Phylum Echinodermata
Ophiuroidea, Brittle stars

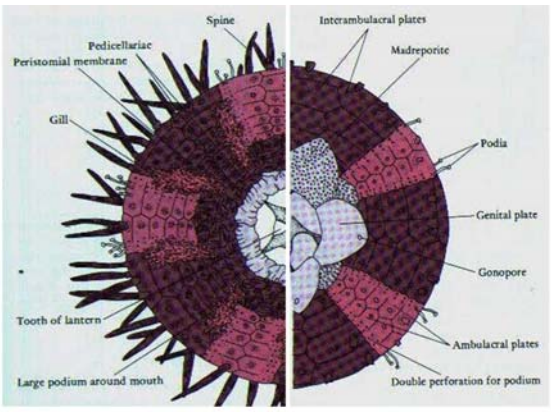

Amphilycus scripta


ดาวเปราะกัลปังหา, Ophiothela danae

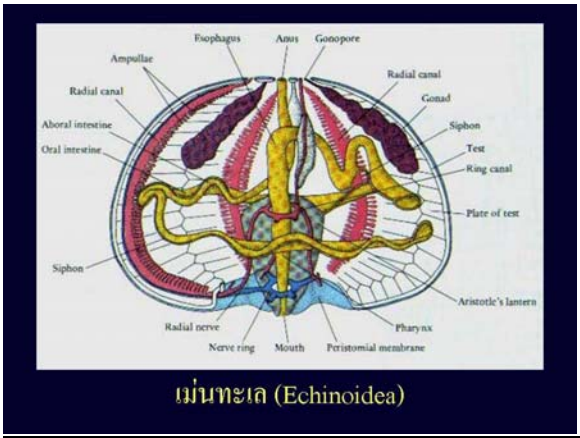

ดาวเปราะหนามใหญ่, Macrophiothrix longipeda

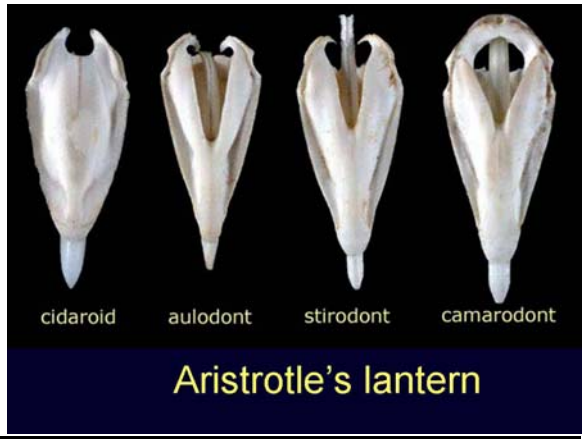

Ophiarachnella gorgonia


Phylum Echinodermata
Echinoidea, Sea urchin, sand dollars, heart urchins
©2007 MBARI


Spine
Pedicellariae
Peristomial membrane
Gill
Tooth of lantern
Large podium around mouth
Interambulacral plates
Madreporite
Podia
Genital plate
Gonopore
Ambulacral plates
Double perforation for podium

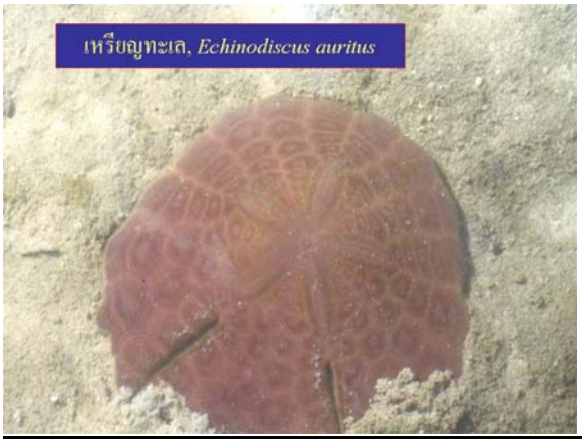
Annex 9


Esophagus
Anus
Gonopore
Ampullae
Radial canal
Radial canal
Gonad
Aboral intestine
Siphon
Oral intestine
Test
Ring canal
Plate of test
Siphon
Aristotle's lantern
Radial nerve
Pharynx
Nerve ring
Mouth
Peristomial membrane
เม่นทะเล (Echinoidea)


cidaroid
aulodont
stirodont
camarodont
Aristrotle's lantern


เม่นดำหนามยาว


เม่นหนามลาย


เหรียญทะเล, Echinodiscus auritus


เม่นหัวใจ, Lovenia elongata

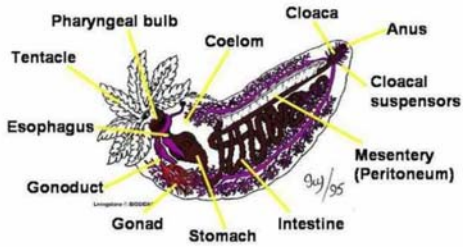

เม่นหัวใจ, Brissus latecarinatus


Phylum Echinodermata
Holothuroidea, Sea cucumber
Sea Cucumber Photo by David Robel 1997

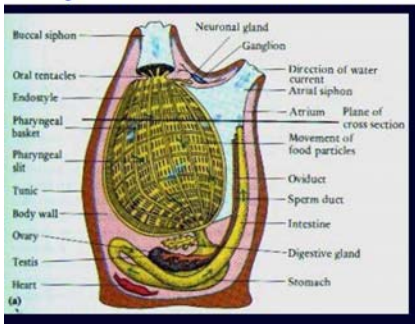
Annex 9

Phylum Echinodermata

Holothuroidea, Sea cucumber



Phylum Urochordata



Ascidian, sea squirts

Phylum Urochordata

Ascidian, sea squirts

Annex 9


Phylum Urochordata
เพรียงหัวหอมเดี่ยว


เพรียงหัวหอมเดี่ยวสีเหลือง


เพรียงหัวหอมเดี่ยวสีส้ม


เพรียงหัวหอมเดี่ยวขาวใส

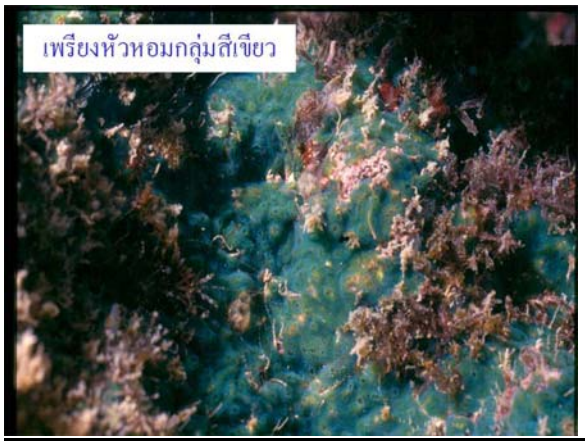

เพรียงหัวหอมเดี่ยวฟ้าใส


เพรียงหัวหอมกลุ่มสีม่วง


เพรียงหัวหอมกลุ่มสีขาว


เพรียงหัวหอมกลุ่มสีส้ม

Annex 9



Invertebrate Preservation

3 steps includes

1. Anaesthetization : relax and set specimen

2. Fixation: Fix specimen, stop microorganism and cells process

3. Preservation: permanent preservation

Anaesthetization : methods

- **Simple methods:** Oxygen starvation, Carbon dioxide excess, Freezing, Ethyl alcohol
- **Formaldehyde method**
- **Magnesium chloride (MgCl2. 6H2O)** ~ 73 g/ DW 1 litre
- **Magnesium sulfate (MgSO4)** 10% in sea water
- **Menthol**

Fixation : Formalin 4-10 %

3. **Preservation** for permanent preserve specimen

Preservation chemicals: 2 most popular chemicals

1. Formaldehyde or formalin

-Change protein in cytoplasm become big molecule and non water soluble kill micro-organisms and non color change

- Commercial grade is 37-40 % concentration, should dilute to 4-10 % by calculated from 37-40 %

- Formalin is acid and not useful for calcareous animal skeleton, it should neutralized by using Borax

Annex 9

2. Ethanol or Ethyl alcohol

- Alcohol is useful for many animals but not useful for animal with fat
- The most popular is Ethanol and Isopropanol
- Non-toxic to human
- Normal concentration for preservation is 70%

Annex 10: Arrangement of Activities and Sample Sorting

By Dr. Natinee Sukramongkol

Annex 10

Arrangement of Activities Onboard M.V. SEAFDEC 2

17 to 19 October 2010

Area of the Sampling Sites

General Information

Title:	Research Methodologies for the Study on Impact of Fishing to Deep-Sea Ecosystem
Period:	17-19 October 2010
Vessel:	M.V. SEAFDEC 2
Area:	Continental slope area of Brunei waters (Zone 3 and 4)
Sea depth:	Two main depth strata 100-200m and 200-300m
Type of: bottom	Muddy (generally)

Activities onboard M.V.SEAFDEC 2

1. Topographic survey using Hydro-acoustic Echo sounder
2. Sampling gear operations (target on demersal fishes and benthic fauna)
   - 2.1 Beam Trawl = 2 operations
   - 2.2 Agassiz Trawl = 2 operations
   - 2.3 Deep-sea traps = 1 operations
3. Sorting specimens, Tagging, Preservation, Data record
4. Seabed survey by towing underwater VDO camera

Day 1 (17)	Day 2 (18)	Day 3 (19)
Topographic Survey #1	Topographic Survey #2	Seabed survey: Towing under-water VDO camera (all hand)
Beam trawl op.01 & Sorting #1	Beam trawl op.02 & Sorting #2	
Shooting Deep-sea trap (all hand)	Topographic survey#3	
	Agassiz Trawl op.01 & Sorting #3	
	Topographic Survey #4	
	Agassiz Trawl op.02 & Sorting #4	
	Hauling Deep-sea trap & Sorting (all hand)	

Group Assignment-I

*Participants (16 persons) will be separated into four groups and rotation for sorting specimens

Op. no.	Fishes	Crustaceans	Mollusk	Other invertebrate
	Dr.Konishi	Dr.Chittima	Ms.Natinee	Dr.Sumaitt
#1	Group 1	Group 2	Group 3	Group 4
#2	Group 2	Group 3	Group 4	Group 1
#3	Group 3	Group 4	Group 1	Group 2
#4	Group 4	Group 1	Group 2	Group 3

Group Assignment-II

**Each group is requested to present the results from the survey on 20th October 2010

Group #1	Group #2	Group #3	Group #4
1. Mr. Pirote	1. Mr. Han Win	1. Mr. Remar	1. Mr. Nguyen
2. Mr. Jamil	2. Mr. Bram	2. Mr. [illegible]	2. Mr. Bidin
3. Mr. Sheikh	3. Ms. Desi	3. Mr. Sayan	3. Mr. Sukchai
4.	4.	4.	4.

Sampling Procedure

Onboard sampling (washing over sieve)

↓

Sorted by species, preliminary identification

↓

Assigning TAG to specimens

↓

Number and Weight of specimens recording (logsheet)

↓

Photography

↓

Preservation

Annex 10


Some photograph of fishes and benthic fauna
during 21 Sep. to 13 Oct. 2010

Annex 11A: Bottom Topographic Survey

By Ms. Penchan Laongmanee

Training Workshop on Research Methodologies for the Study on Impact of Fishing to Deep-Sea Ecosystem

16-20 October 2010, Negara Brunei Darussalam

Bottom Topographic Survey

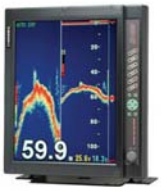
Penchan Laongmanee
& Sukchai Arnupapboon

Sounding

The first primitive maps were rendered from successions of single soundings produced by lowering weighted lines into the water and noting when the tension on the line slackened, indicating the ocean floor. The depth was then measured by the amount of line paid out.

http://oceanexplorer.noaa.gov/explorations/02fire/background/seafloor_mapping/seafloor.html

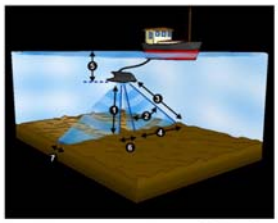
Single beam echo sounder

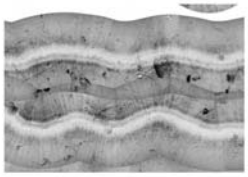


Echo sounding is the technique of using sound pulses directed from the surface or from a submarine vertically down to measure the distance to the bottom by means of sound waves

http://en.wikipedia.org/wiki/Echo_sounding

Side-scan Sonar

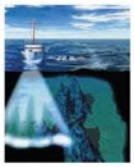


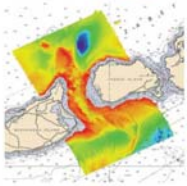


The sound frequencies used in side-scan sonar usually range from 100 to 500 kHz; higher frequencies yield better resolution but less range

http://en.wikipedia.org/wiki/Side-scan_sonar

Multibeam echo sounders (MBES)

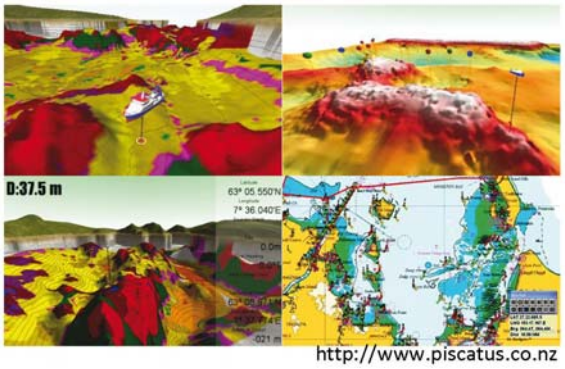




Multibeam echo sounders (MBES), like other sonar systems, transmit sound energy and analyze the return signal (echo) that has bounced off the seafloor or other objects. Multibeam sonars emit sound waves from directly beneath a ship's hull to produce fan-shaped coverage of the seafloor.

http://www.nauticalcharts.noaa.gov/hsd/multibeam.html

Multibeam echo sounder in commercial fishing boat

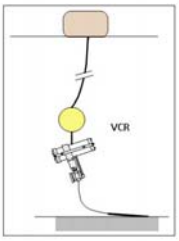


http://www.piscatus.co.nz

Substrate type: Under water VDO camera

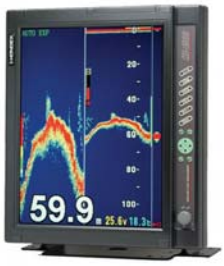
Watanabe and Kitagawa, 2003

Substrate type: Under water VDO camera, camera



Annex 11A

Bottom topographic survey activity



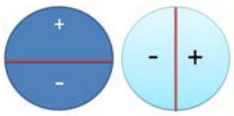
	A	B	C	D
1	longitude	latitude	depth	
2	119.805	16.4798	-469	
3	119.804	16.478	-469	
4	119.803	16.476	-468	
5	119.802	16.4738	-469	
6	119.802	16.4722	-470	
7	119.801	16.471	-471	
8	119.801	16.4703	-471	
9	119.801	16.4693	-471	
10	119.8	16.4682	-471	
11	119.799	16.467	-471	
12	119.798	16.4655	-471	
13	119.797	16.4643	-472	
14	119.797	16.4633	-473	
15	119.796	16.4632	-473	

Arranging position data

- Calculate recorded number from Echo Sounder convert to decimal number

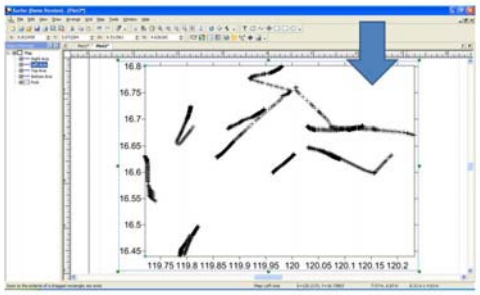
Degree + (min/60) = decimal number

- Longitude → N → +, S → -
- Latitude → E → +, W → -
- Depth → -



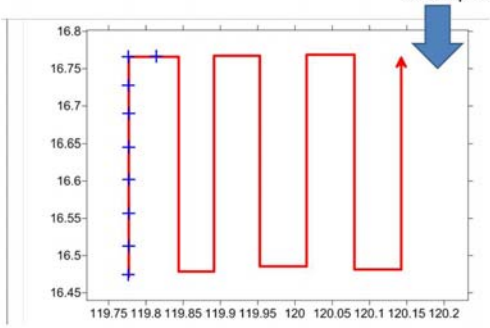
Position of data

Scatter data

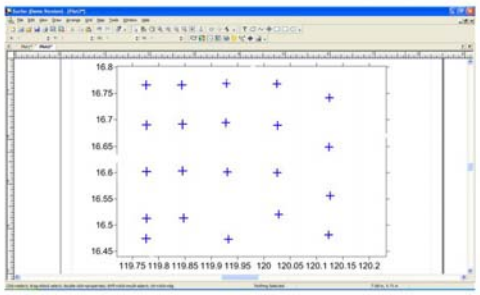


Position of data

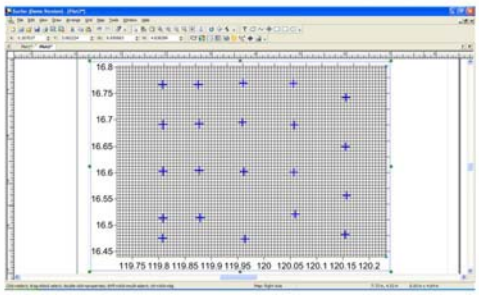
On ship track



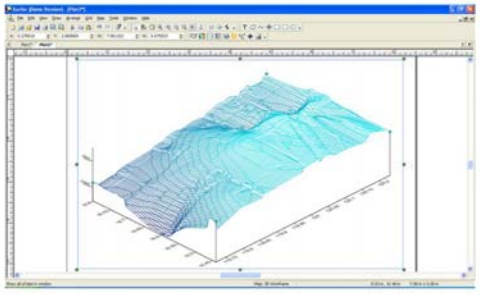
Position of data



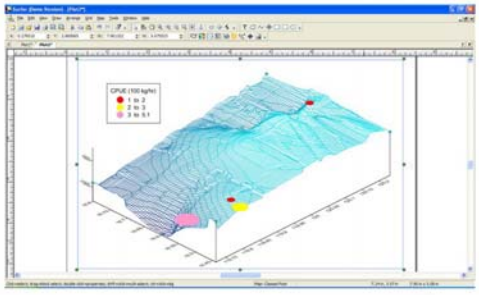
Add data position → gridding method



3D wire frame map

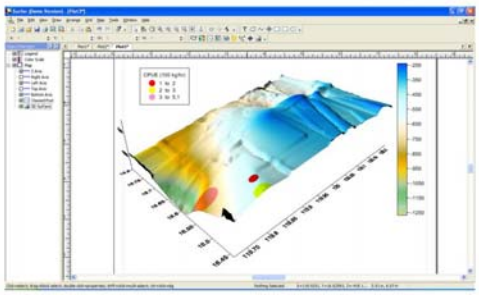


Overlay with CPUE



Annex 11A

3D surface map



Annex 11B: Seafloor Survey Using Underwater VDO Camera

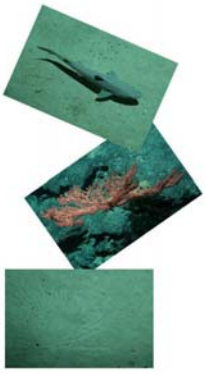
By Mr. Sukchai Arnupapboon

Annex 11B

Objective

- To identify benthic resources and seafloor features for biodiversity or relationships between benthic and seafloor features

15



Seafloor Survey Using Underwater Video Camera

Sukchai Arnupapboon
and
Penchan Laongmanee

SEAFDEC's facilities

ROV
Remote Operated Vehicle

TUV
Towed Underwater Video

2

ROV

- Advantage
  - Able to operate in submarine obstruction area
  - Able to close-up examination on seafloor
  - Able to collect benthic samples

3

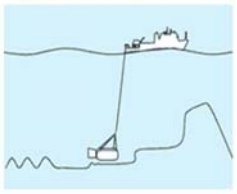
ROV

- Disadvantage
  - -Difficult to fix distance from objective
  - -Difficult to employ in strong current area
  - -Radios operation (Sampling is non-random)

4

TUV

- Without frame



- With frame

5

TUV

- Advantage
  - Able to survey large expanse of seafloor
  - Allow precise density measurement of interest

6

TUV

- Disadvantage
  - Unable to focus on objective
  - Could damage by obstruction or constant by seafloor topography

7

Annex 11B

TUV component

- Underwater camera
- Metal frame
- Monitor display
- Light
- GPS

8

Video camera features

- Wide angle lens, deepest recess to protect against accidents
- Lens is auto focus
- Inert-gas filled electronic compartment

9

Metal frame design

- Towable on the seafloor
- Adjustable camera angel
- Flexible tow angle

10

Metal frame design

- Towable on the seafloor

11

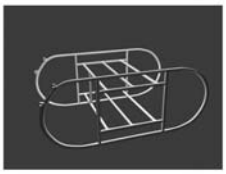
Metal frame design

- Adjustable camera angle

12

Metal frame design

- Flexible tow angle



13

Survey method and analysis

- Mount video camera to metal frame normally above seafloor 50-100 cm
- Deploy to seafloor when ship moving slowly
- Keep tow speed at 1 knot or below as far as possible and record the condition of the seafloor along a series of transects
- Record onto data file and copy to DVD and review with slow motion
- Count the feature of interest

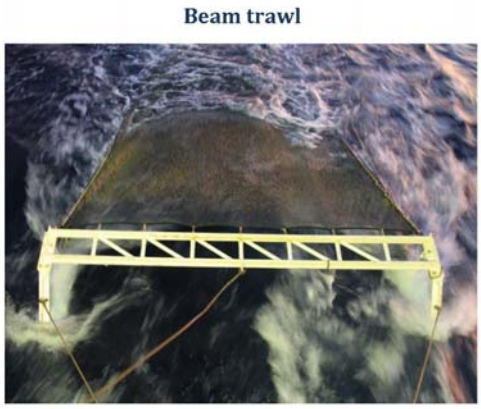
14

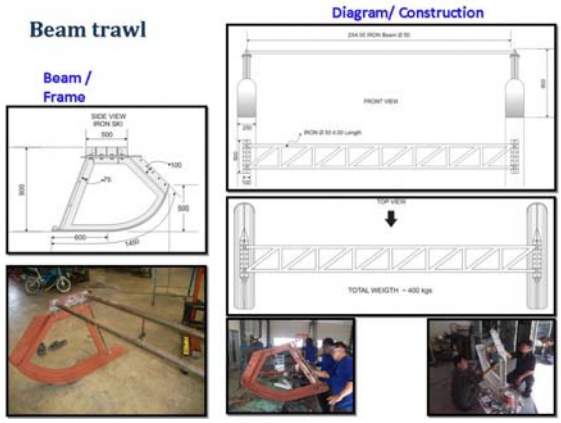
Annex 12: Fishing gear and method: Agassi trawl, Beam Trawl and Deep-Sea Trap

By Mr. Sayan Promjind and Mr. Narong Ruangsivakul

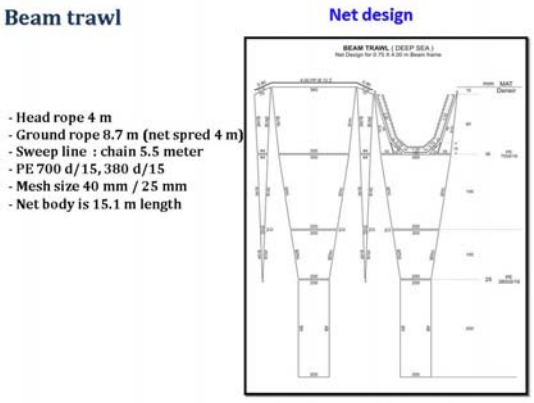
Annex 12


Deep-sea fishing gears and method
for the Training Workshop
on Research Methodologies for the Study
on Impact of Fishing to Deep-Sea Ecosystem
16-20 October 2010
Brunei Darussalam
Beam Trawl
Agassiz Trawl
Deep sea Trap
Sayan Promjinda
Narong Ruangsivakul
Capture Fisheries Technology Division
Southeast Asian Fisheries Development Center
Training Department

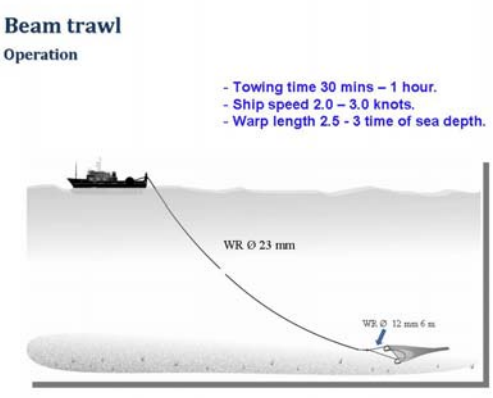

Beam trawl


Beam trawl
Diagram/ Construction
Beam /
Frame


Beam trawl
Diagram
Beam /
Frame


Beam trawl
Net design
- Head rope 4 m
- Ground rope 8.7 m (net spred 4 m)
- Sweep line : chain 5.5 meter
- PE 700 d/15, 380 d/15
- Mesh size 40 mm / 25 mm
- Net body is 15.1 m length


Beam trawl
Gear preparation

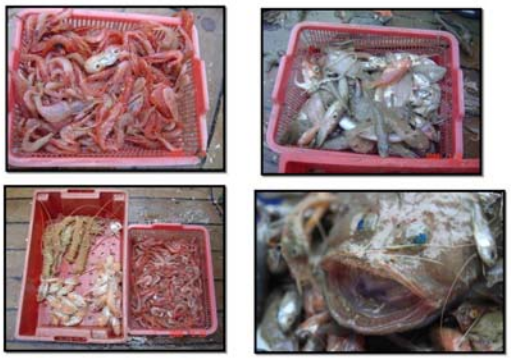

Beam trawl
Operation
- Towing time 30 mins – 1 hour.
- Ship speed 2.0 – 3.0 knots.
- Warp length 2.5 - 3 time of sea depth.
WR Ø 23 mm


Beam trawl
Sampling sorting

Annex 12

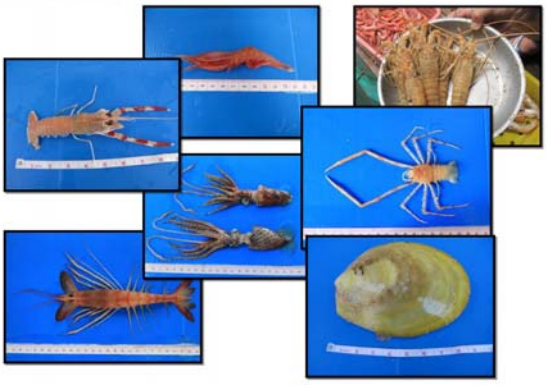
Beam trawl

Specimens



Beam trawl

Specimens



Agassiz trawl

The Agassiz trawl is used to collect organisms, particularly invertebrates, living on the ocean bottom.

www.kc-denmark.dk

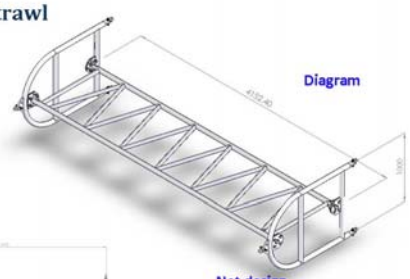
Agassiz trawl

Construction

Frame Ø 380 mm 4 meter STT pipe
- Weight ~ 200 kg

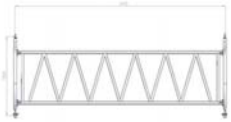
Agassiz trawl

Diagram



Net design

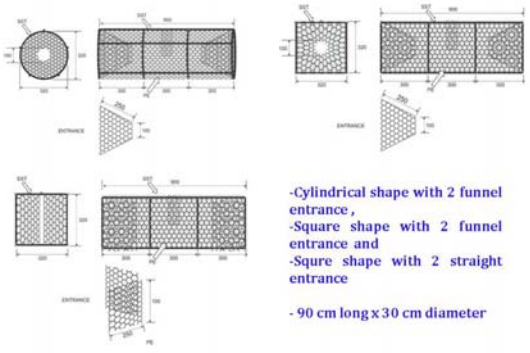
- Head rope 4 m
- Ground rope 4 m
- PE 700 d/15,
- Mesh size 40 mm
- Net body is 15 m length



Deep SeaTraps

Deep Sea Trap

Construction



-Cylindrical shape with 2 funnel entrance ,
-Square shape with 2 funnel entrance and
-Squre shape with 2 straight entrance

- 90 cm long x 30 cm diameter

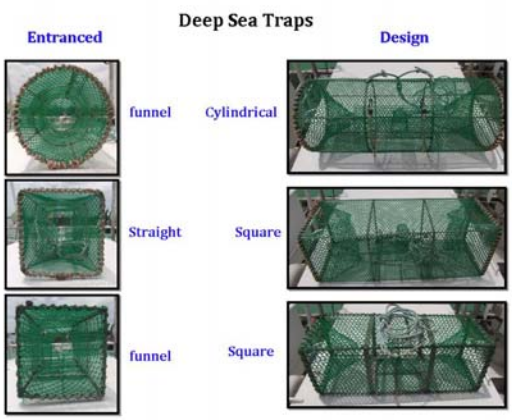
Deep Sea Traps

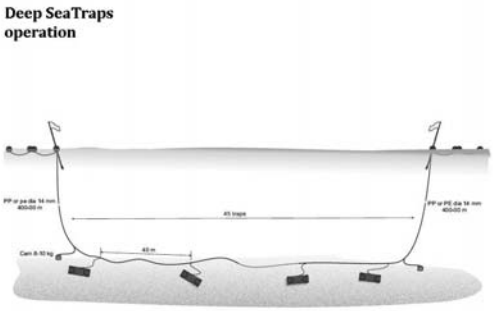
Construction

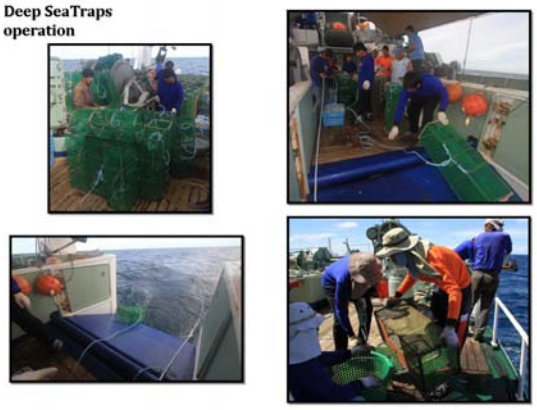
- Stationary fishing gear with
- baits inside

- Made plastic net, STT frame

Annex 12


Deep Sea Traps
Entranced
Design
funnel
Cylindrical
Straight
Square
funnel
Square


Deep SeaTraps
operation


Deep SeaTraps
operation


Deep SeaTraps
The specimens from DST


THANK YOU FOR
ATTENTION