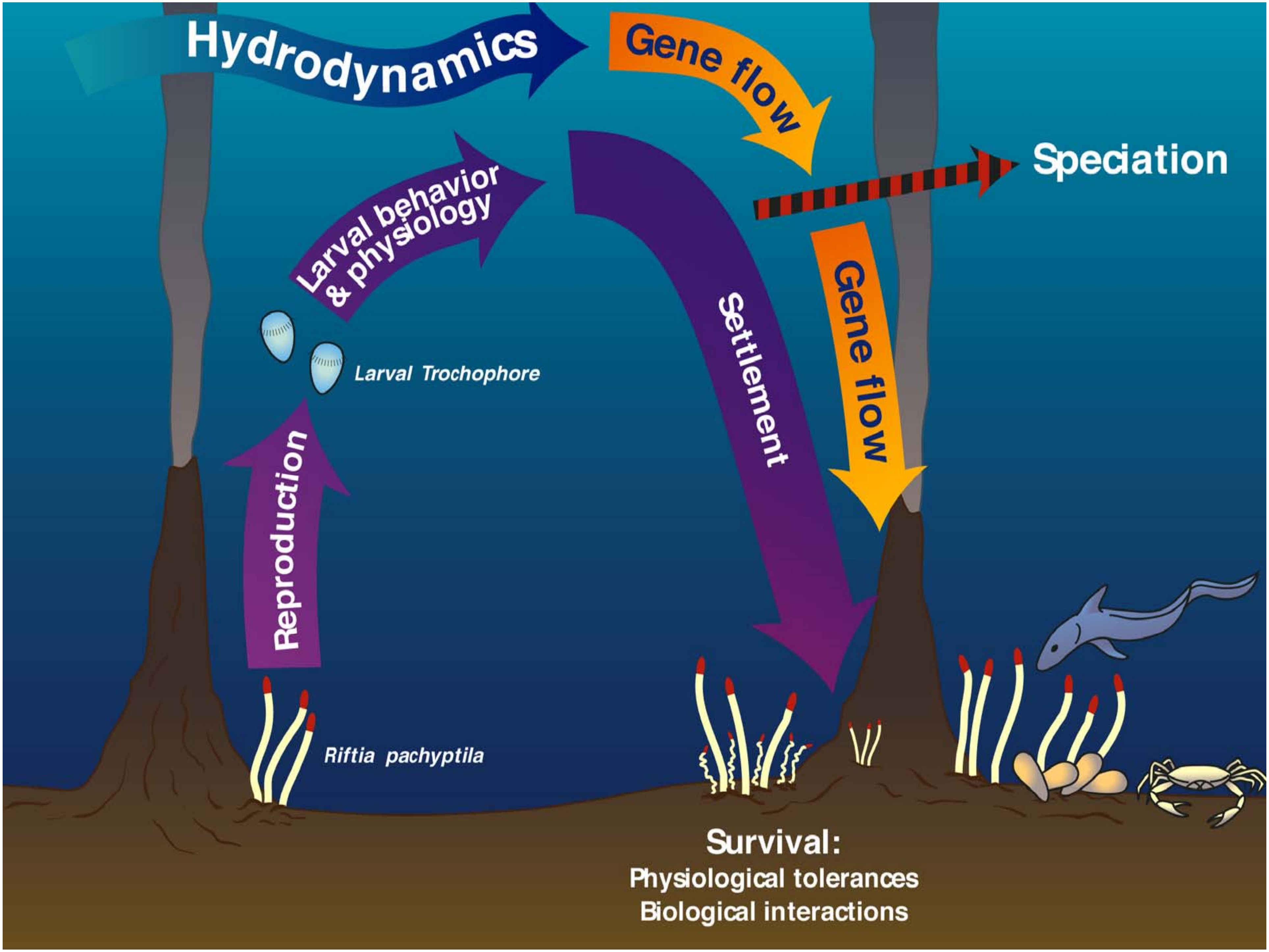
International Seabed Authority

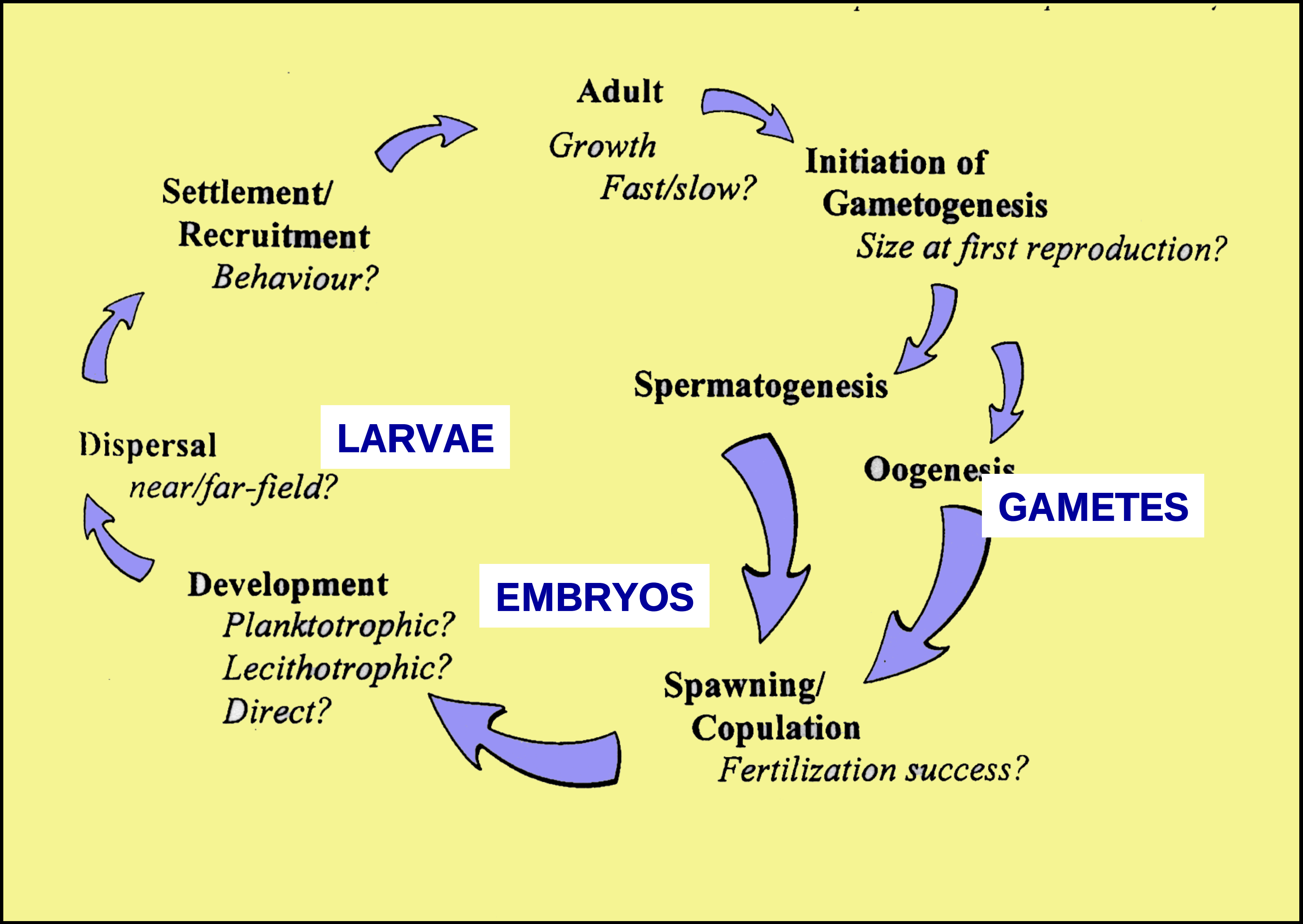
Seamount Genetics: Enabling the Understanding of Biodiversity, Connectivity, Evolution, and Endemism

Timothy M. Shank
Biology Department
Woods Hole Oceanographic Institution

International Seabed Authority
Workshop on Deep Seabed Cobalt-rich crusts and the Diversity and Distribution Patterns of Seamount Fauna.
Kingston, Jamaica March 27-31 2006


Hydrodynamics
Gene flow
Speciation
Larval behavior
& physiology
Settlement
Gene flow
Larval Trochophore
Reproduction
Riftia pachyptila
Survival:
Physiological tolerances
Biological interactions

Life History Strategies

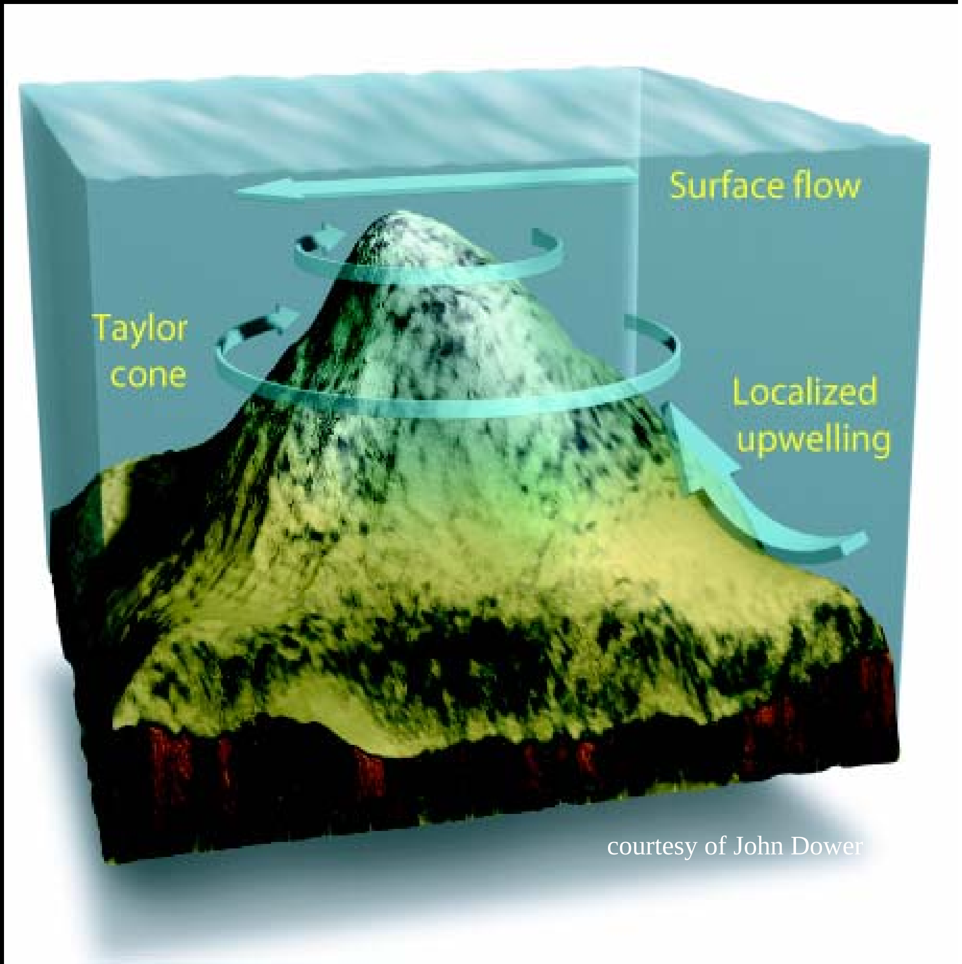


Physical Oceanographic Processes

Important parameters affecting the flow around seamounts:

Coriolis parameter (geographic location)
Horizontal steady forces
Stratification conditions
Seamount geometry

Localized upwelling interacts with steady surface flows form anti-cyclonic eddies or Taylor columns and caps- these can aggregate mid-water fauna and larvae above a seamount; also inhibit the dispersal of larvae away from source population.



Ex. Great Meteor Seamount (Dower and Mackas 1996)

In contrast, populations can experience enhanced connectivity and gene flow via rectified flows along seamount chains, like the Northwest Hawaiian Seamount chain

Seamounts are a great place to look at the interaction of physical and biological processes- ideal laboratories to study isolation and speciation

Critical Questions Well-Suited to Genetic Approaches

• What are the key processes controlling seamount diversity, population connectivity, evolution, and endemism on seamounts?

• To what degree are seamount communities isolated, regulated by physical processes (extrinsic) vs. reproduction, life-history strategies (intrinsic)?

• What is the role of seamounts in creating and maintaining marine biodiversity? Are seamounts globally significant centers of speciation?

• Do seamount habitats provide stepping-stones for dispersal between distant populations?

• How does habitat loss (e.g., due to fisheries and mining activities) affect genetic diversity and the maintenance of species through gene flow?

Genetic Approaches to Assess Diversity, Connectivity and Evolution of Seamount Fauna

Molecular systematics

- address questions of taxonomic boundaries, identification and determination via comparison of morphological characters with genetic markers (DNA barcoding & identifying species-specific larvae)

Phylogenetics

- address questions of inter-specific/generic/familial relatedness; formation and radiation of diversity; speciation events; evolution of ecological adaptations of species and groups (interspecific approach)

Population genetics (includes phylogeography or historical population migrations)

- seeks to understand the history, formation, and persistence of the factors that inhibit, promote, and control dispersal; mechanisms of larval dispersal; identification of dispersal barriers, biogeographic boundaries, and stock structure for conservation and management (intraspecific approach)

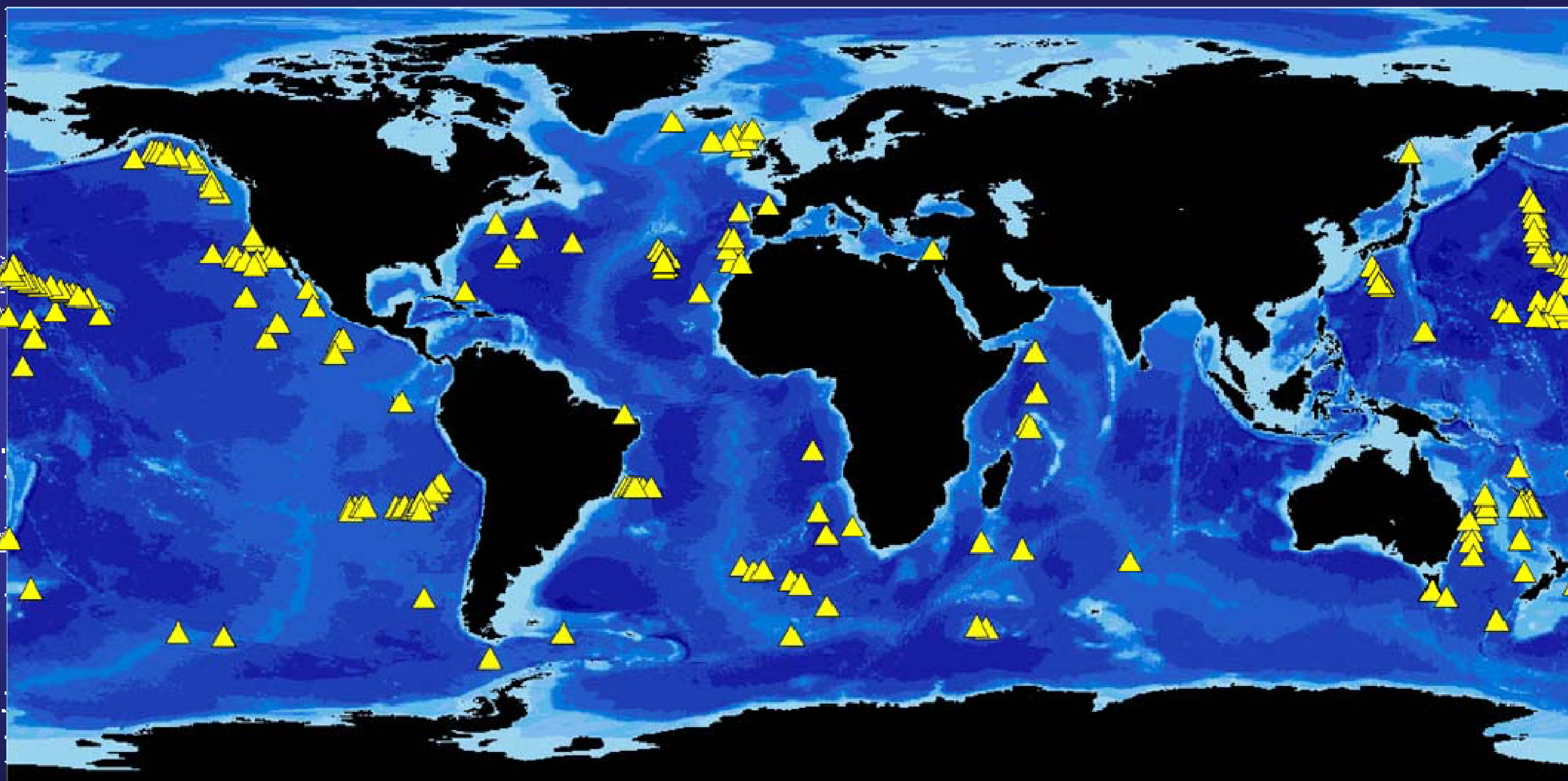
"Community genetics"

- assessing host and associate relationships; co-evolution among species and populations; including congruence of dispersal patterns and mechanisms

Genetic Tools for Inferring Gene Flow and Isolation

- Allozymes
  - multiple, independent, codominant loci; relatively easy; low cost
  - not at the DNA level, subject to selection, state characters
- Mitochondrial DNA
  - relatively easy; maternally inherited; effectively haploid; non-recombining; modest cost; amenable to genealogical analysis
  - *Low variability in deep-water corals provides little utility*
- Nuclear DNA sequences
  - amenable to genealogical analysis;
  - diploid; recombination; start-up time may be considerable
- AFLPs (Amplified Fragment Length Polymorphisms)
  - nuclear markers; can get 100s of loci relatively easily
  - dominance; recombination; state characters; start-up time is considerable; mutation models not available
- DNA microsatellites (and Expressed Sequence Tags)
  - nuclear; can get dozens of loci relatively easily
  - recombination; state characters; start-up time and cost$ is great; problem with homoplasy in geographical studies; mutation must be taken into account in gene flow models

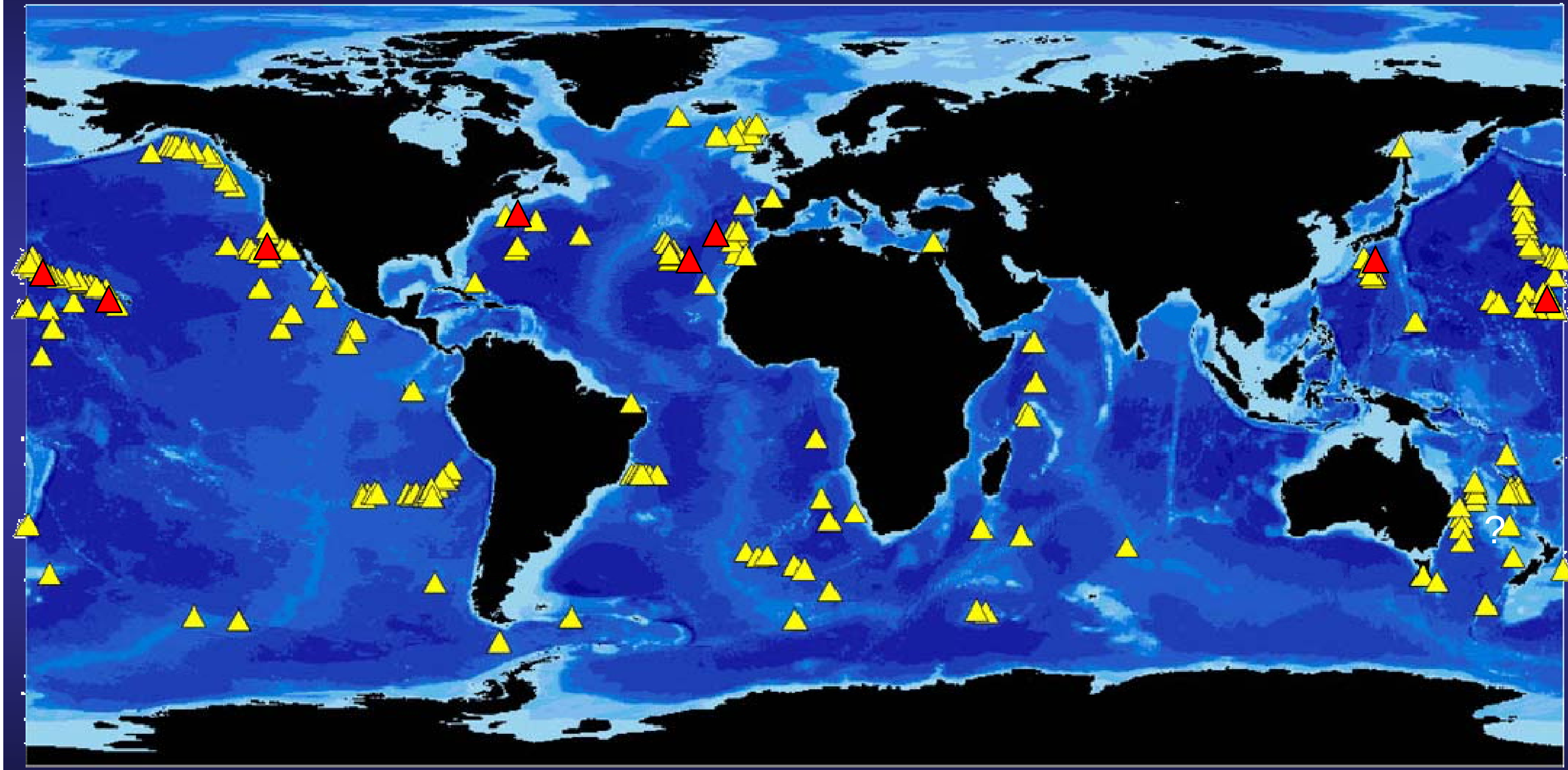
Sampled Seamounts



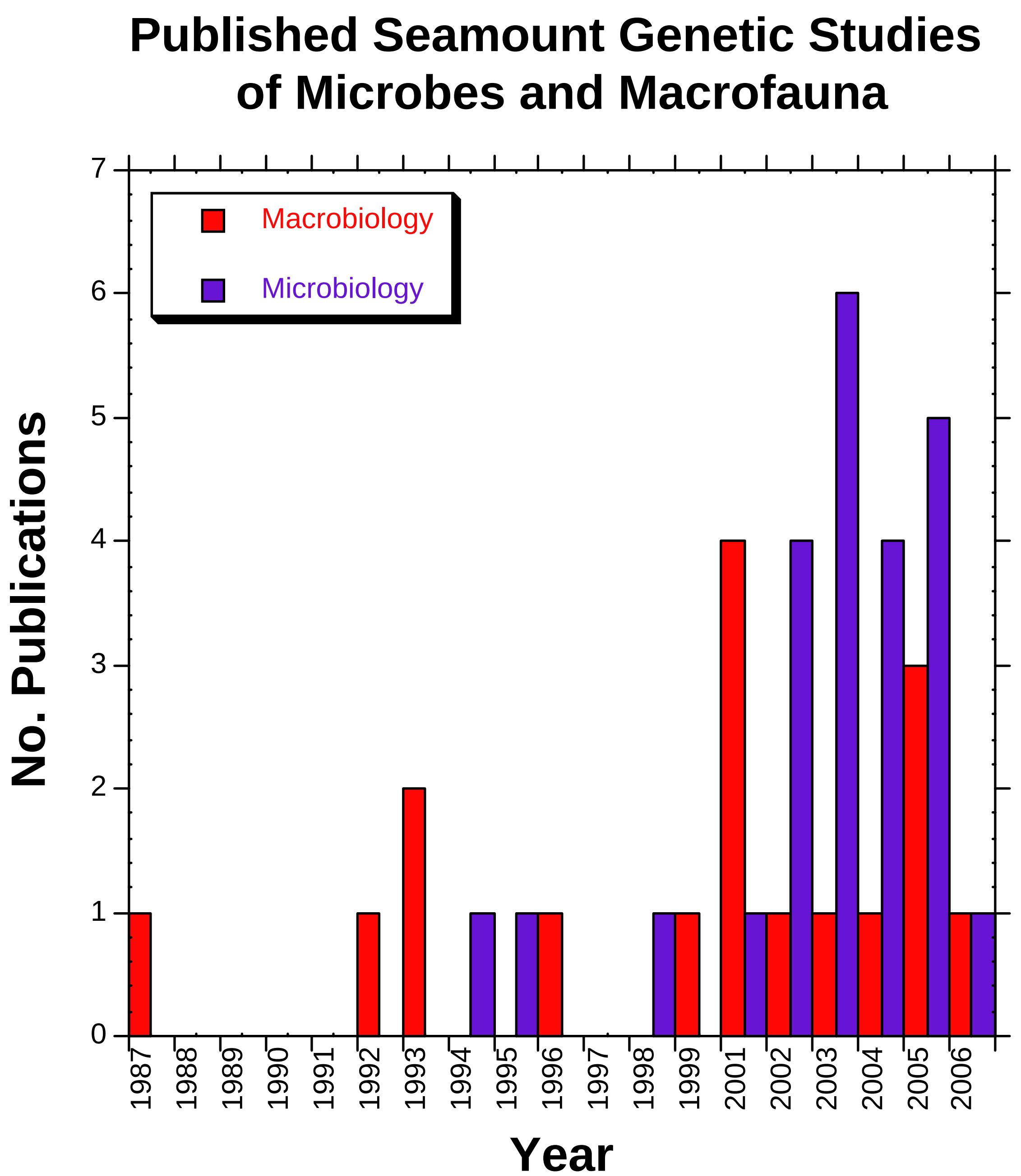
~250 sampled, out of >50,000 (>1000 m), “millions” (<1000 m)

(stolen from Malcolm Clark)

Sampled Seamounts



▲ = published genetic studies



Published Seamount Genetic Studies
of Microbes and Macrofauna
Macrobiology
Microbiology
No. Publications
0
1
2
3
4
5
6
7
1987
1988
1989
1990
1991
1992
1993
1994
1995
1996
1997
1998
1999
2001
2002
2003
2004
2005
2006
Year

Genetics of Seamount-Related Fauna

Selected Phylogenetic and Systematic Examples:

- "What are the deeper evolutionary (and systematic) relationships among the Anthozoa?"

  France et al., *Mol Mar Biol Biotechnol* (1996); Song and Won., *Korean J. Biol Sci* (1997)

  Berntson et al., *Molecular Phylogenetics and Evolution* (1999) -18S rRNA nuclear gene sequences

- "What are the primary phylogenetic relationships within the Octocorallia?"

  Berntson et al., *Marine Biology* (2001) -18S rRNA nuclear gene sequences
  Sánchez et al., *Molecular Phylogenetics and Evolution* (2003)
  Insertions-deletions (INDELS) and ribosomal RNA secondary structure

- "What are the evolutionary relationships among scleractinian corals? Are they aligned with taxonomic classifications?"

  La Goff-Vitry et al., *Molecular Phylogenetics and Evolution* (2004)
  16S rDNA gene sequences

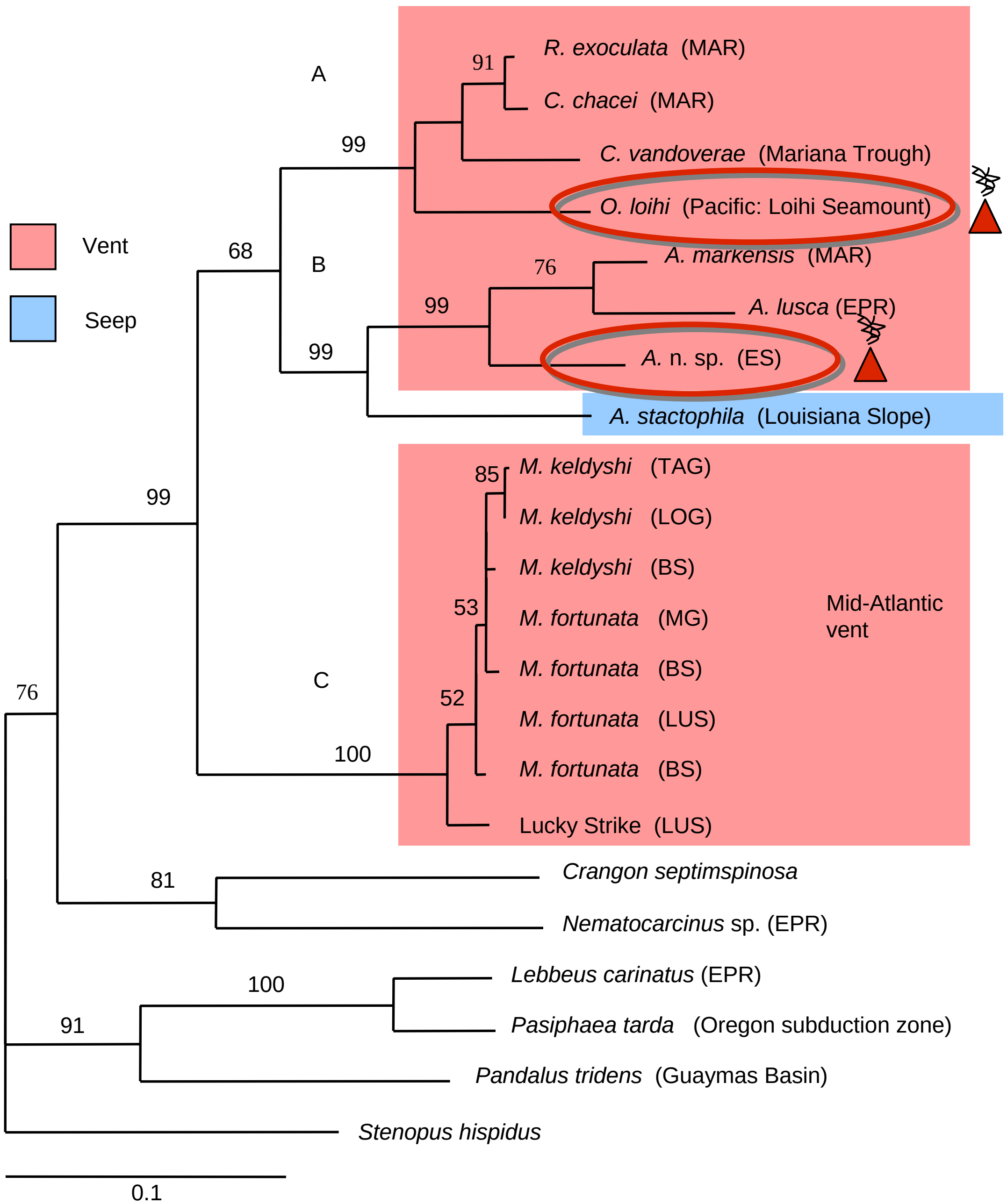
- "Do combined molecular and morphological systematics resolve difficulties in anthazoan classification?"

  Won et al., *Coral Reefs* (2001) -18S rRNA nuclear gene sequences and 41 morphological characters

Hydrothermal vent and seep-endemic shrimp

Shank TM, Halanych K, Black M, Lutz RA, Vrijenhoek RC (1999) Miocene radiation of deep-sea hydrothermal vent shrimp (Caridea: Bresiliidae): evidence from mitochondrial cytochrome oxidase subunit I. Molecular Phylogenetics and Evolution 12: 244-254

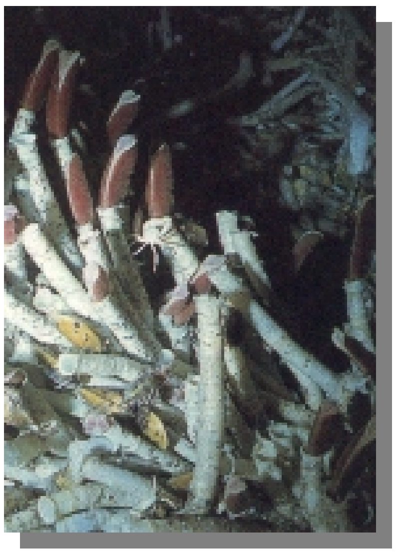
Molecular Phylogenetics -evolutionary relationships

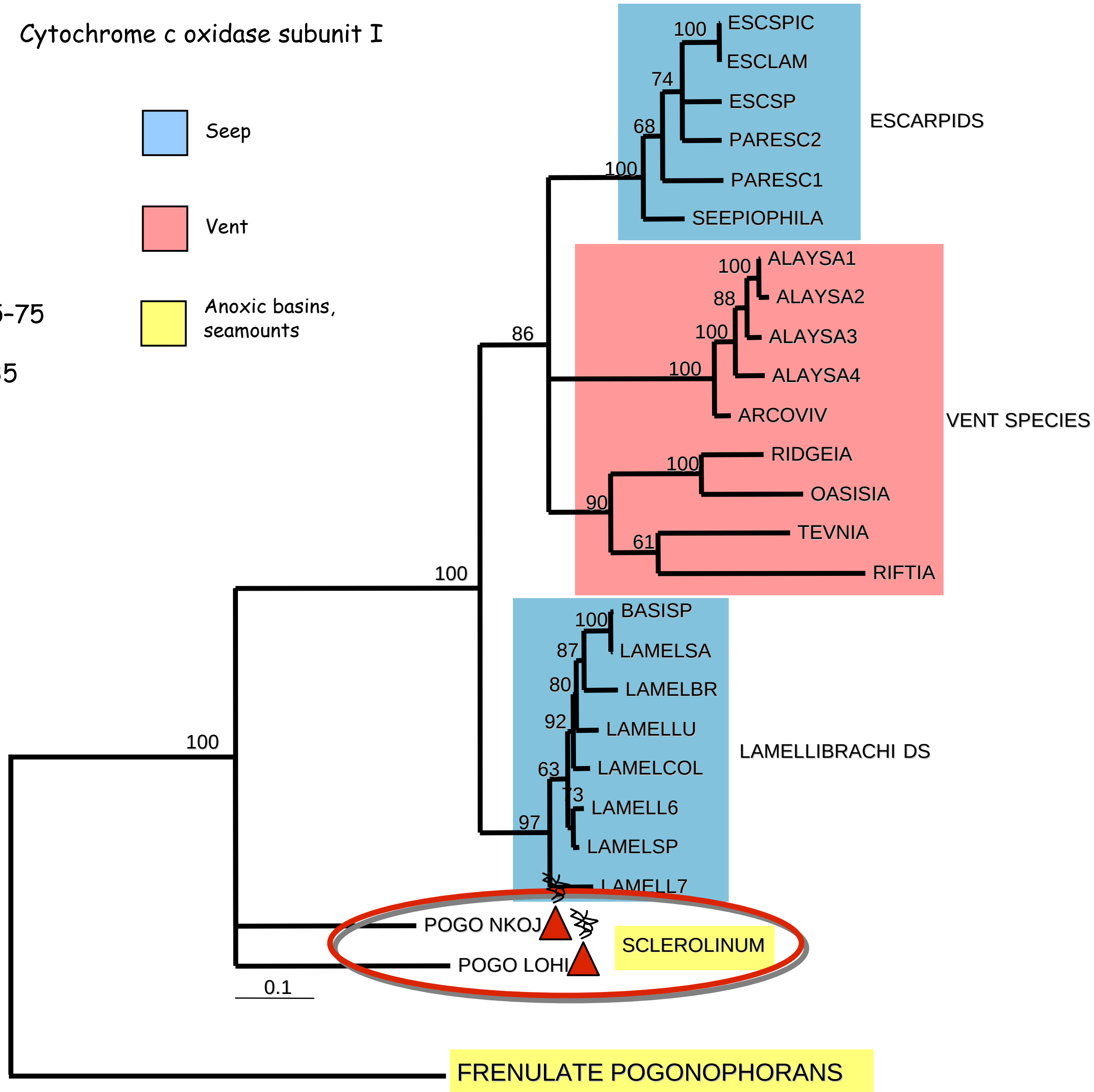


Vent and seep siboglinid tubeworms

Molecular Phylogenetics -evolutionary relationships

Compiled from:
Black *et al.* (1997) Marine Biology 130: 141-149
Halanych *et al.* (2001) Biological Bulletin 201: 65-75
McMullin *et al.* (2003) Symbiosis 34: 1-41
Kojima *et al.* (2003) Marine Biology 142: 625-635





Molecular Systematics -determining diversity

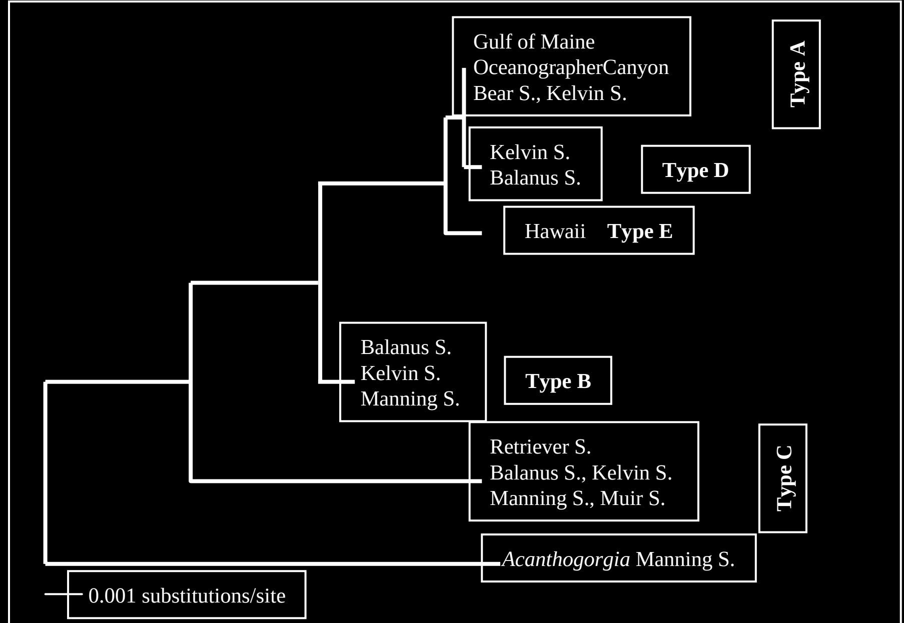
	Number of Species		Practicing Taxonomists			
	Number of Species	Number deeper than 50 m	Total	Deep Water	Molecular Approach	Comments
Scleractinia	1482	615 (41.5%)	10	3.5	1	2 students, 1 admin.
Zoanthidea	2 (of 300)	2	2	2	1	*Gerardia*, 1 student
Antipatharia	*225	169 (75%)	2	2	0	1 student
Octocoralllia	*2850	2140 (75%)	16	6.5	6	2 students, 2 retired
Stylasteridae	246	220 (89%)	1	1	1	1 student
Hydractiniidae	3 (of 78)	1	1	0	1	1 student; *Janaria, Hydrocorella, Polyhydra*
Milleporidae	17	0	1	0	0	
TOTAL	4825	3145 (65.2%)	33	15	10	8 students, 2 retired, 1 admin.

* estimate

from S. Cairns

Molecular Systematics -determining diversity

Paramuricea octocoral (New England Seamounts)



Neighbor-joining phylogram (K2 distance) of *Paramuricea* (Octocorallia) based on 1367 bp of aligned *msh1* sequences from 42 individuals (S. France, unpubl. data) showing four haplotypes from the NE Seamounts

(S. France, unpublished data)

Morphology of the microscopic sclerites (the primary character used in octocorallian taxonomy) did not distinguish colonies that had divergent genetic haplotypes, including the most divergent (1.6%).

This suggests that there may be morphologically-cryptic species variation among seamount octocorals and reinforces the observation that octocoral taxonomy and systematics cannot rely solely on morphology of skeletal characters (Sánchez et al. 2003; Wirshing et al. 2005).

BARCODE OF LIFE DATA SYSTEMS

Advancing species identification and discovery through the analysis of short, standardized gene regions

THE ROCKEFELLER UNIVERSITY

Guelph Centre for DNA Barcoding

CONSORTIUM FOR THE BARCODE OF LIFE

www.barcoding**life**.org

The Barcode of Life Initiative is an emerging collaborative effort to accelerate acquisition of and access to knowledge of biodiversity using mitochondrial DNA sequences (Cytochrome Oxidase I gene).

The Barcode of Life Data Systems (BOLD) is an online workbench that aids collection, management, analysis, and use of DNA barcodes.

Total Species Barcodes 96,356 (as of this morning)

NO barcode data from Seamounts exists in this database
(>200 entries will be submitted by the end of 2006!)

Questions in Seamount-"Related" Coral Genetics

Selected Population Genetic Examples:

- "What is the effect of retention and dispersal of larvae on genetic population structure?"

  France and Hoover, *Hydrobiologia* (2002) (Octocorals)

  mitochondrial Cytochrome Oxidase I sequences

- "Are scleractinian populations in the Atlantic genetically isolated, and if so, to what extent?"

  Le Goff-Vitry and Rogers., In: *Cold Water Coral and Ecosystems* (2005)

  Internal Transcribed Spacer (ITS) 1 and 2 nuclear regional; 10 msatellite markers

- "What are the genetic units of precious coral populations, and are they genetically isolated?"

  Baco and Shank, In: *Cold Water Coral and Ecosystems* (2005)

  6 microsatellite markers

Amphipods

Eurythenes gryllus from the crest of 3 bathyal seamounts as well as from the summit and base of Horizon Guyot (Bucklin et al., 1987)

Eurythenes gryllus up to 15cm length

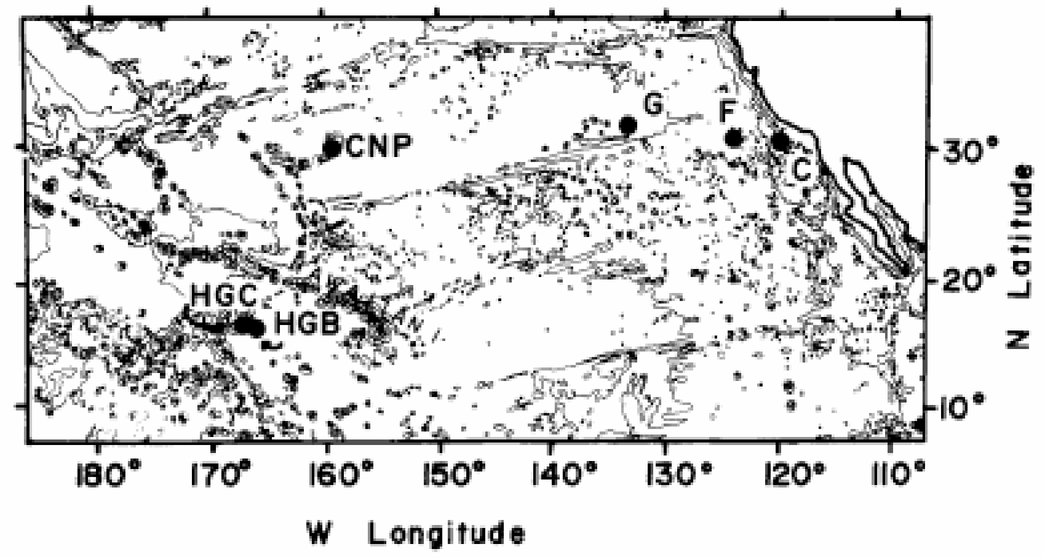


Fig. 1 Seafloor topography along the basin transect showing the locations of basin transect stations (C, F, G and CNP), the Horizon Guyot crest (HGC) station and the station near the base of the seamount (HGB). Exact station locations and particulars are given in SMITH and BALDWIN (1984).

- high gene flow at similar depths
- no gene flow over a 3800m depth range on Horizon Guyot

Fish (Seamount associated)

Armorhead, *Pseudopentaceros wheeleri*, North Pacific Seamounts (Martin et al., 1992)
Mitochondrial DNA, RFLP markers
• Different seamounts do not harbor genetically distinct populations

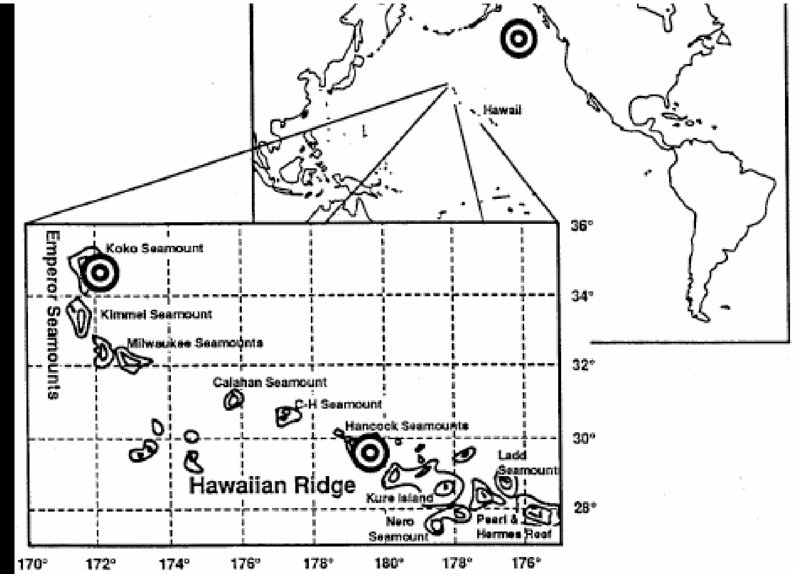


Fig. 1. Map of the Northwestern Hawaiian Islands – Southern Emperor Seamounts showing the location of collection sites for armorheads. Collections were obtained from Koko Seamount, Hancock Seamount, and for pelagic-phase fish from a region bounded by 41–44°N latitude and 157–165°W longitude.

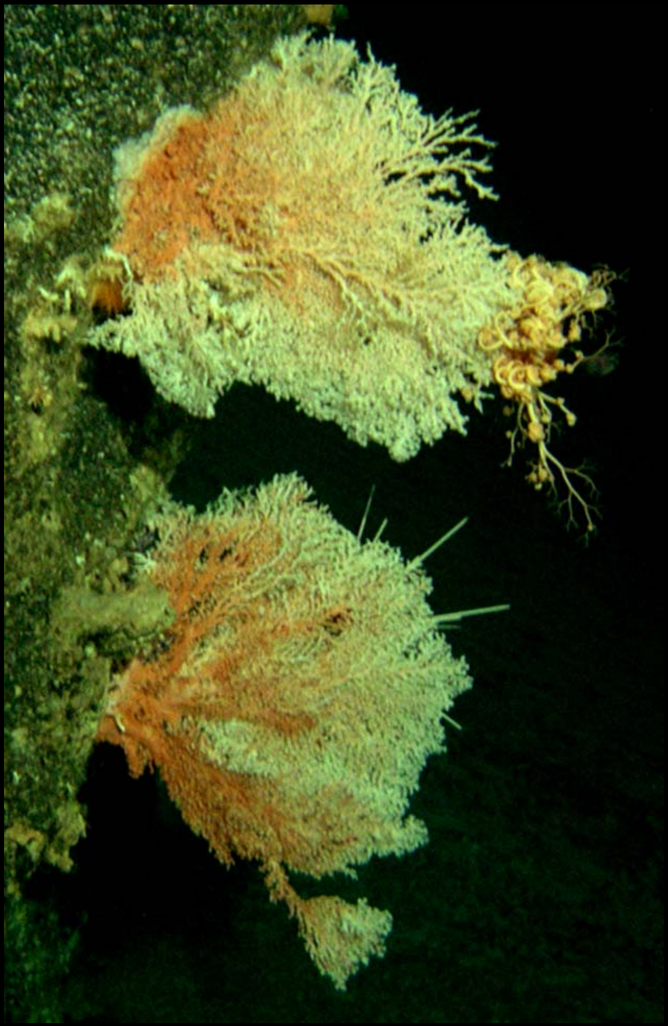
Blackspot seabream, *Pagellus bogaraveo*
N. Atl. & Azores (Stockley et al., 2000), microsatellite markers
• showed little genetic differentiation

Blue-mouth red fish, *Helicolenus dactylopterus*
N. Atl. & Azores (Aboim et. al., 2003; 2005)
microsatellite and mitochondrial DNA
• showed population expansion after population bottleneck

Population genetics- connectivity & isolation

Hawaiian Precious Corals

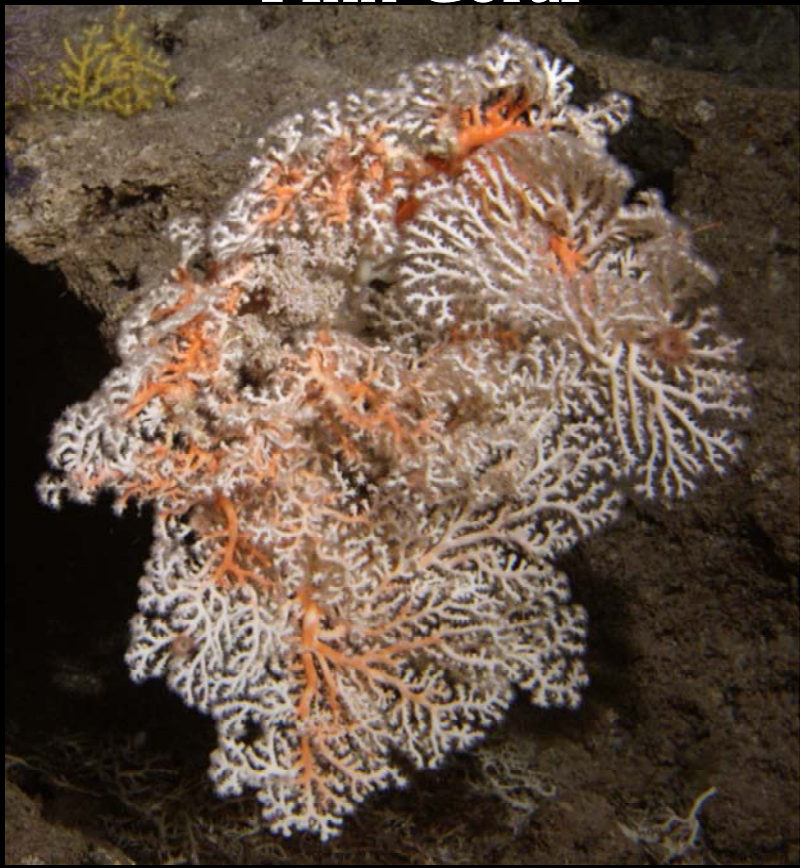
Red Coral



Corallium lauuense (regale)

(300 - 550 m)

Pink Coral



Corallium secundum

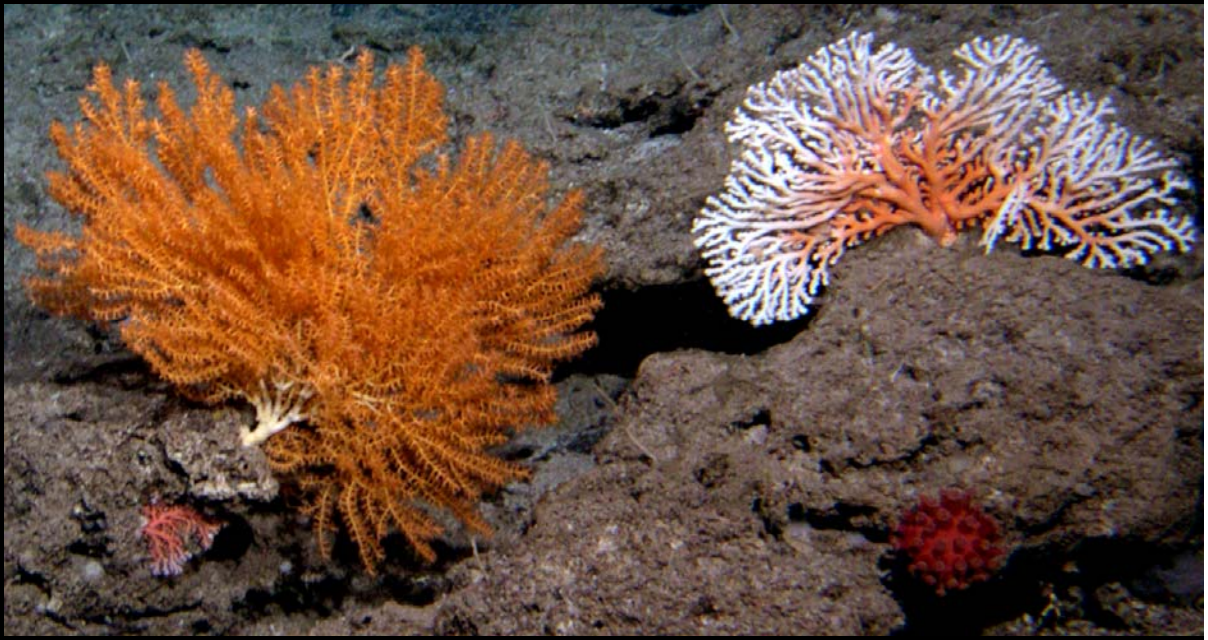
Baco and Shank, 2005


¥ 150000

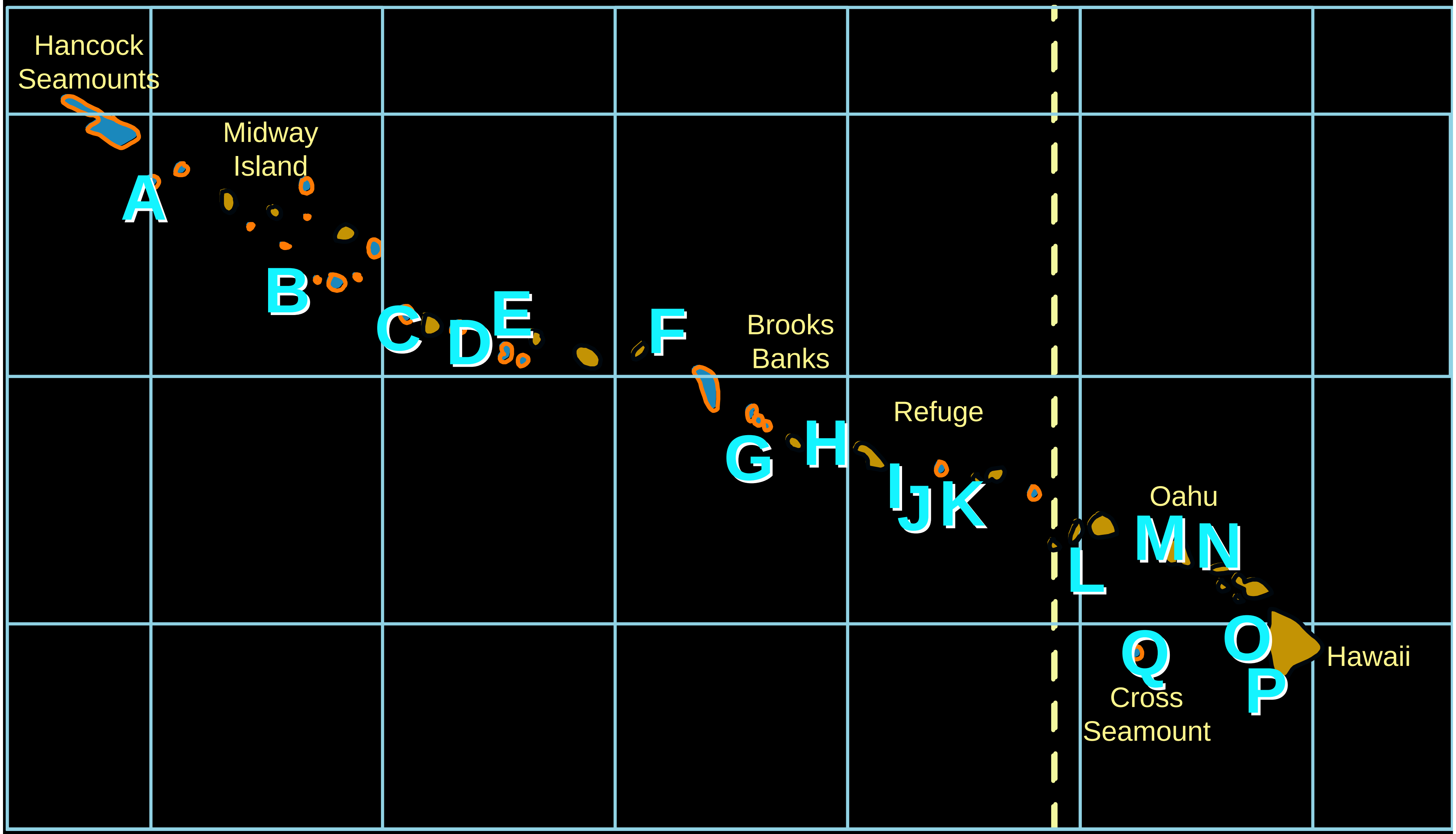

お買得
さんご
シルバーリング ¥10,000
¥ 5,000
¥ 5000
¥ 10000

Hawaiian Precious Corals

- Profitable fishery
- Only one "refuge" in entire Hawaiian chain
- Study of Mediterranean precious corals has shown limited dispersal ability and significant genetic structure between locations

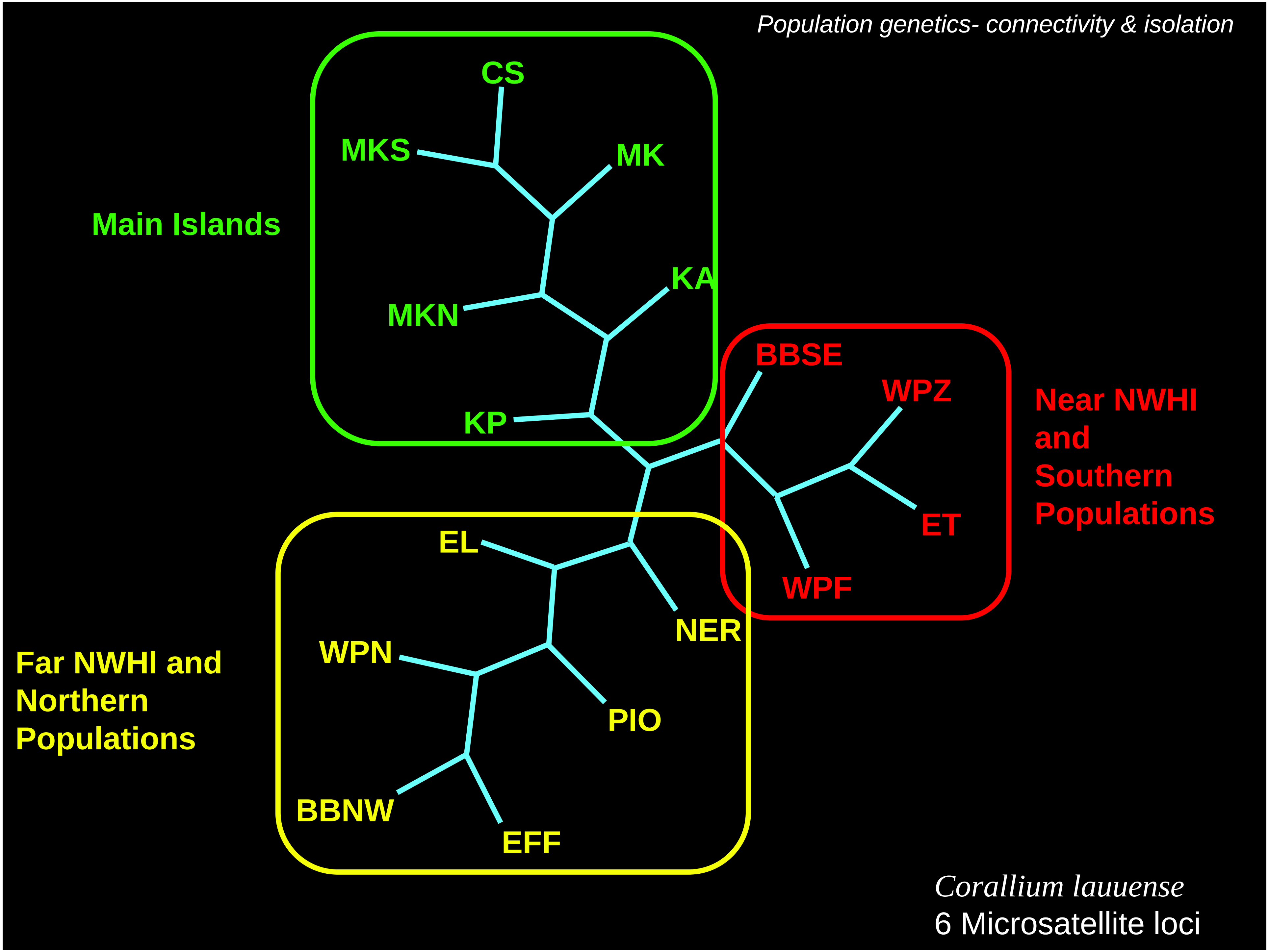


Population genetics- connectivity & isolation

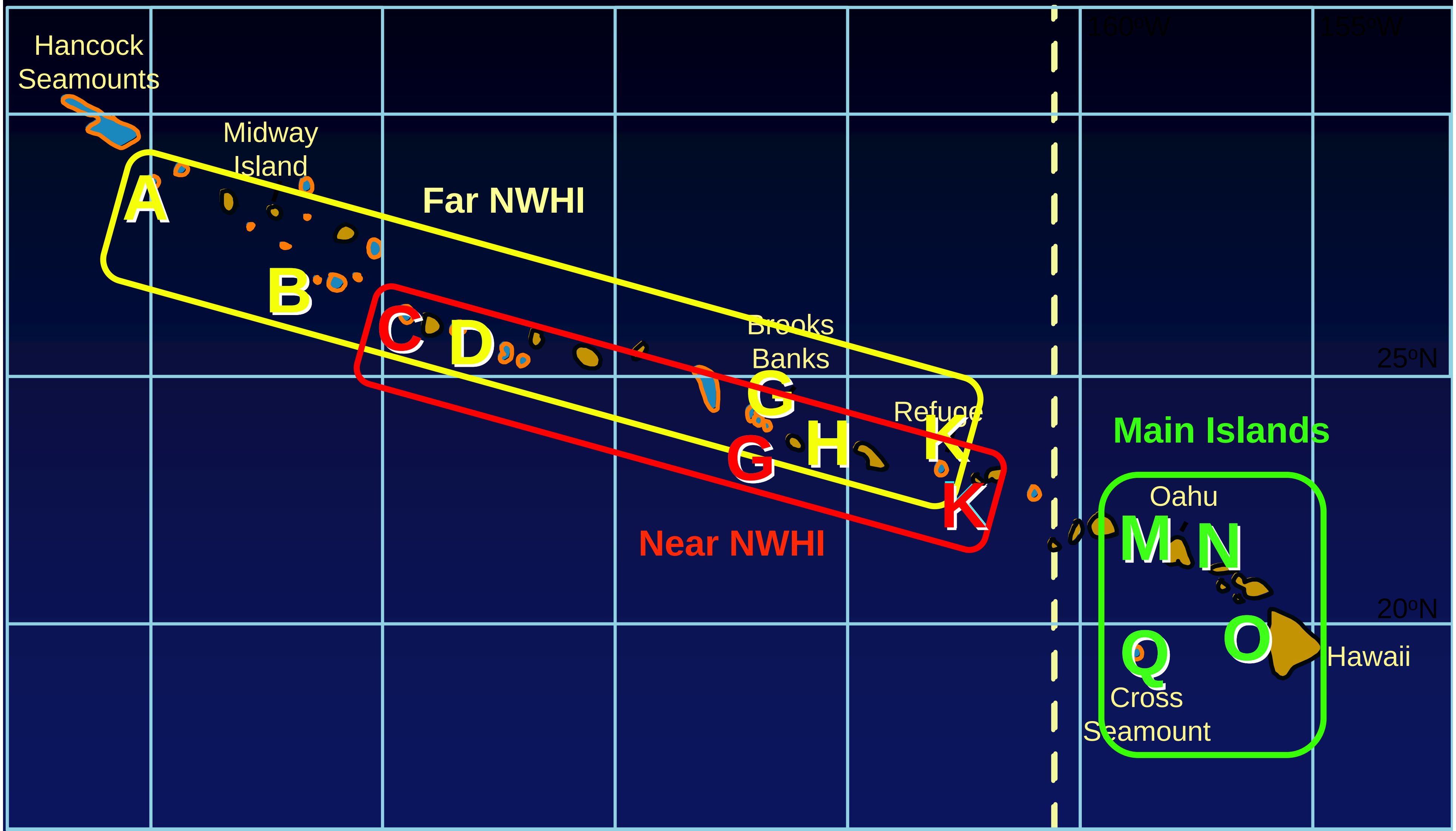


Northwest Hawaiian Islands

Main Hawaiian Islands


Population genetics- connectivity & isolation
Main Islands
CS
MKS
MK
KA
MKN
KP
Near NWHI and Southern Populations
BBSE
WPZ
ET
WPF
Far NWHI and Northern Populations
EL
NER
WPN
PIO
BBNW
EFF
Corallium lauuense
6 Microsatellite loci

Population genetics- connectivity & isolation



Northwest Hawaiian Islands

Main Hawaiian Islands

Conclusions

- **Pink Coral:**

  Revealed genetic structure within “continuous populations”

  No significant structuring between populations on Oahu

  Some genetic structure between pops on Brooks Banks

  Evidence for isolation by distance

  Heterozygote deficiency (lack of diversity) at one locus

- **Red Coral:**

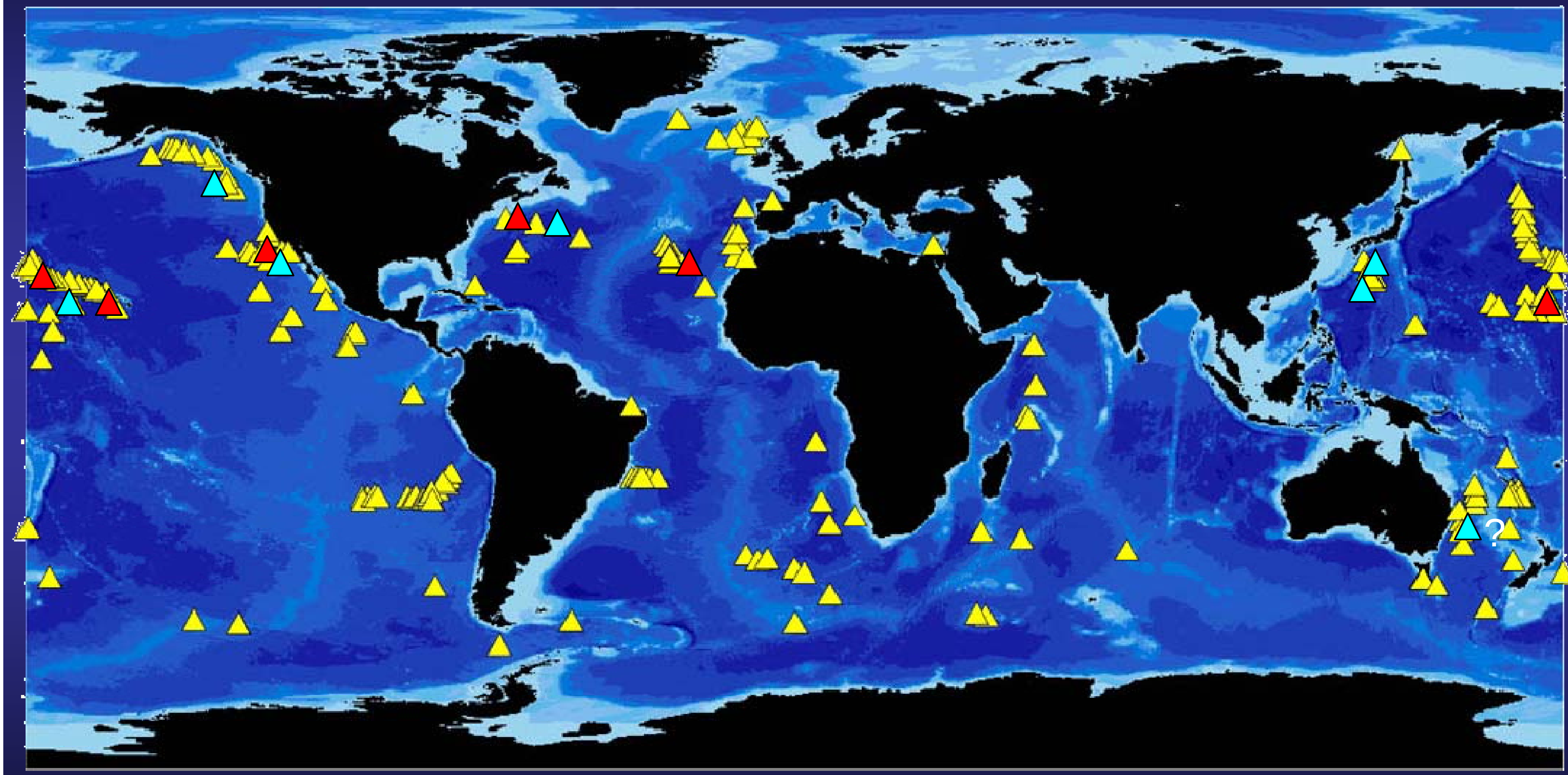
  Heterozygote deficiency at all sites - inbreeding?

  No evidence for isolation by distance

  Kauai population and Bank 8 population isolated from other populations

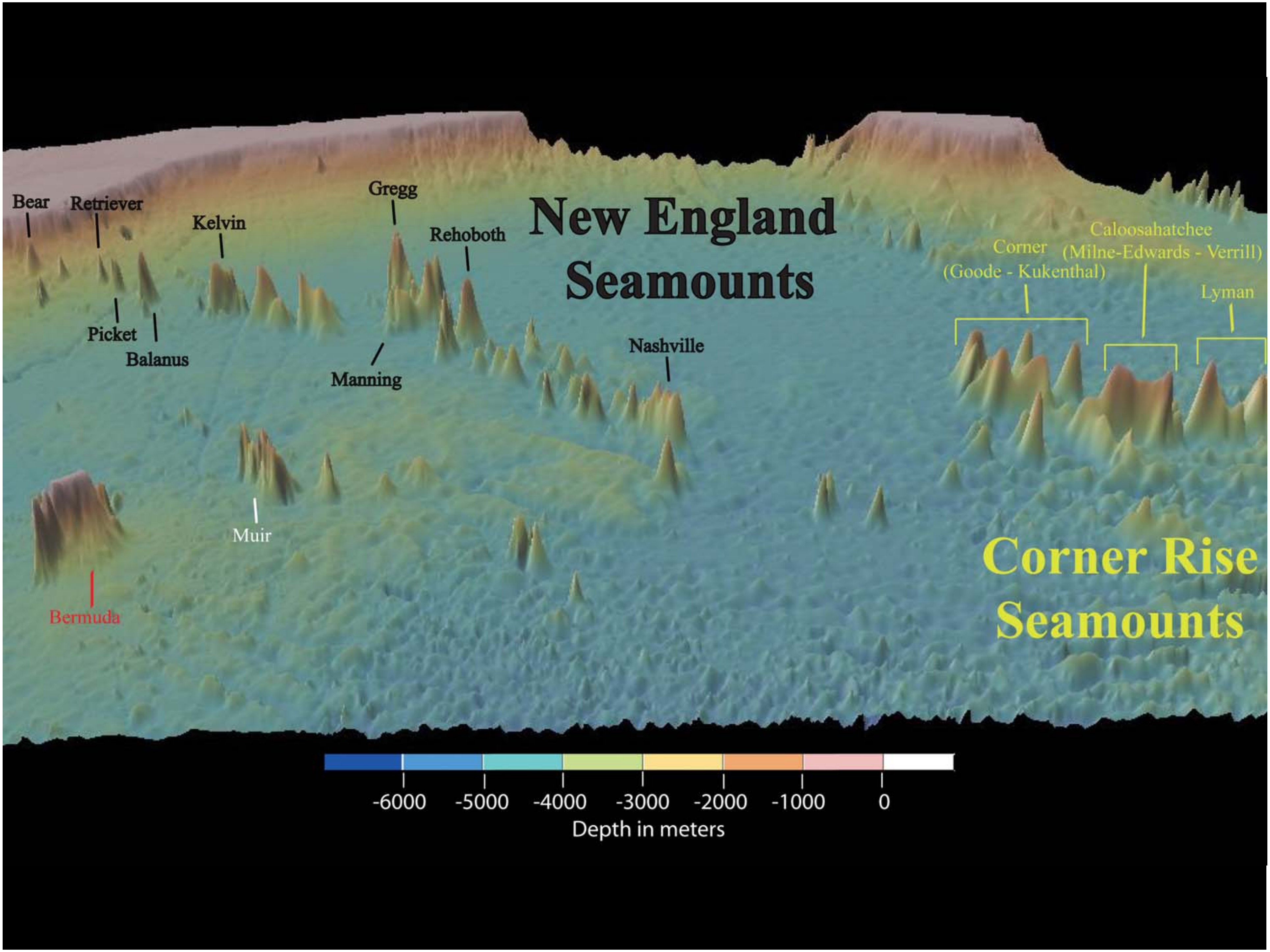
2 sympatric species with different dispersal patterns

Sampled Seamounts



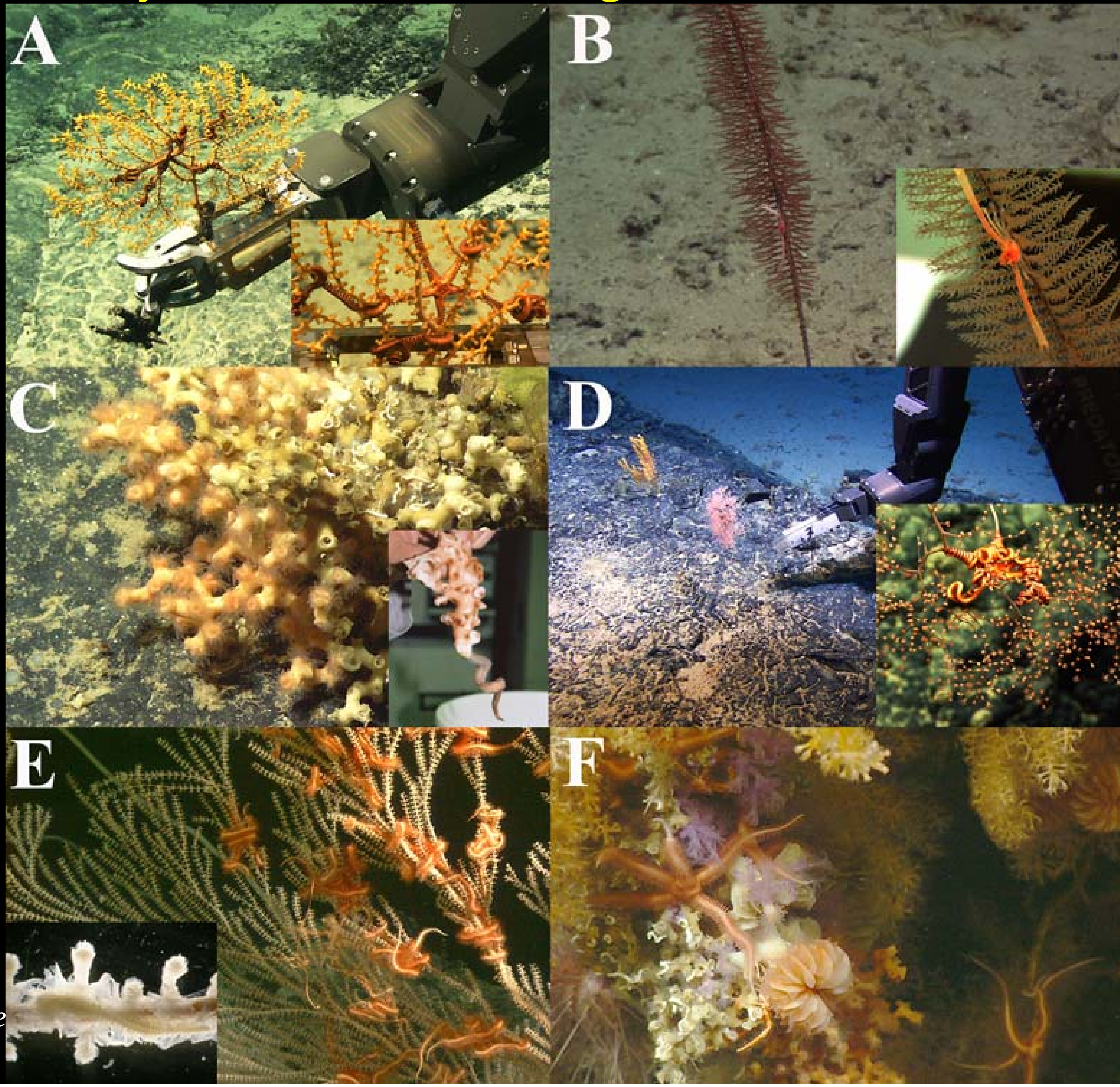
▲ = published genetic studies

▲ = ongoing genetic studies



Bear
Retriever
Kelvin
Gregg
Rehoboth
New England Seamounts
Picket
Balanus
Manning
Nashville
Corner (Goode - Kukenthal)
Caloosahatchee (Milne-Edwards - Verrill)
Lyman
Muir
Bermuda
Corner Rise Seamounts
-6000
-5000
-4000
-3000
-2000
-1000
0
Depth in meters

Host-associate systems on the New England and Corner Rise Seamounts



octocoral *Paramuricea* brittle star *Asteroschema*

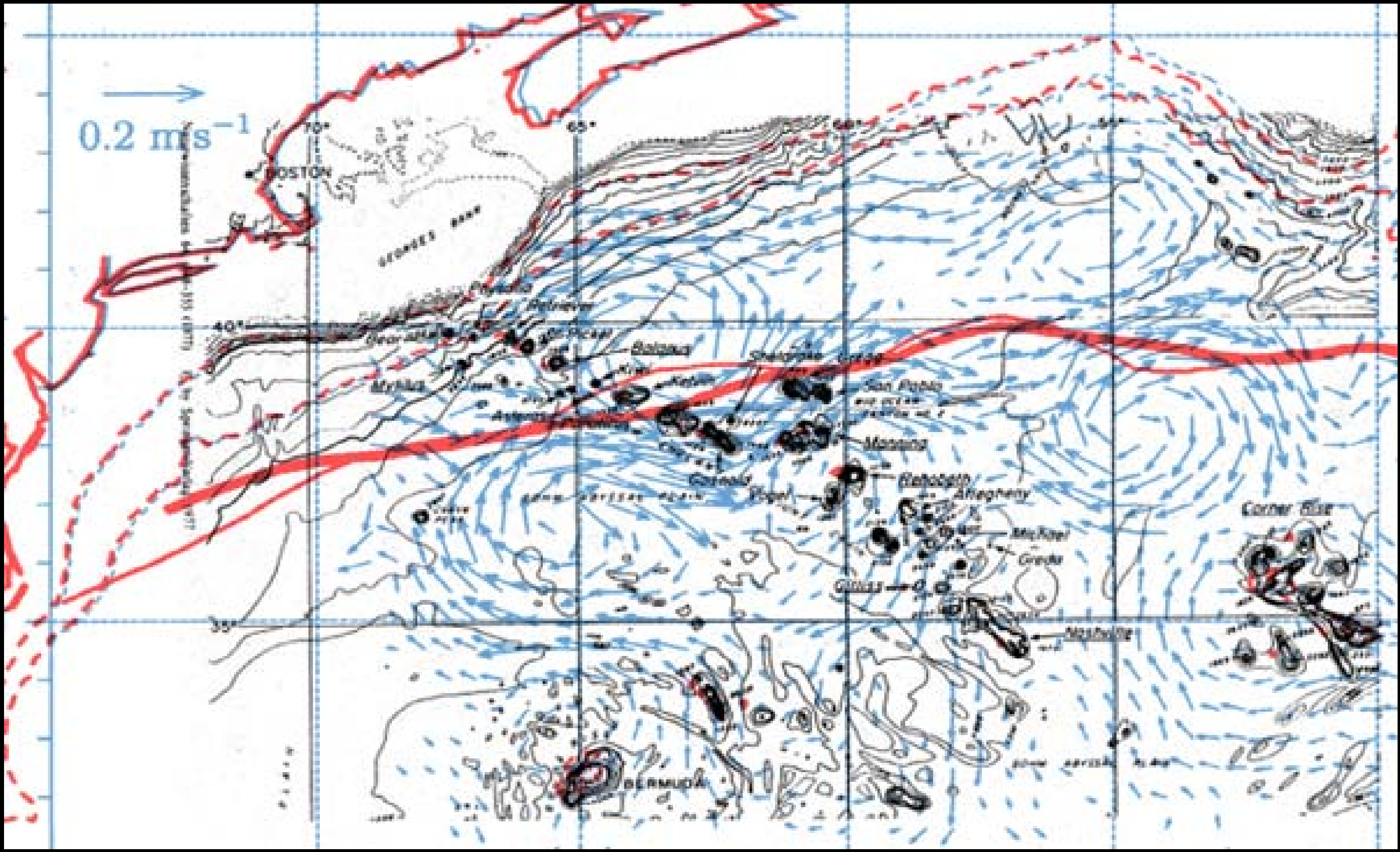
black coral *Parantipathes* & chirostylid crab

scleractinian coral *Lophelia pertusa* & polychaete worm *Eunice*

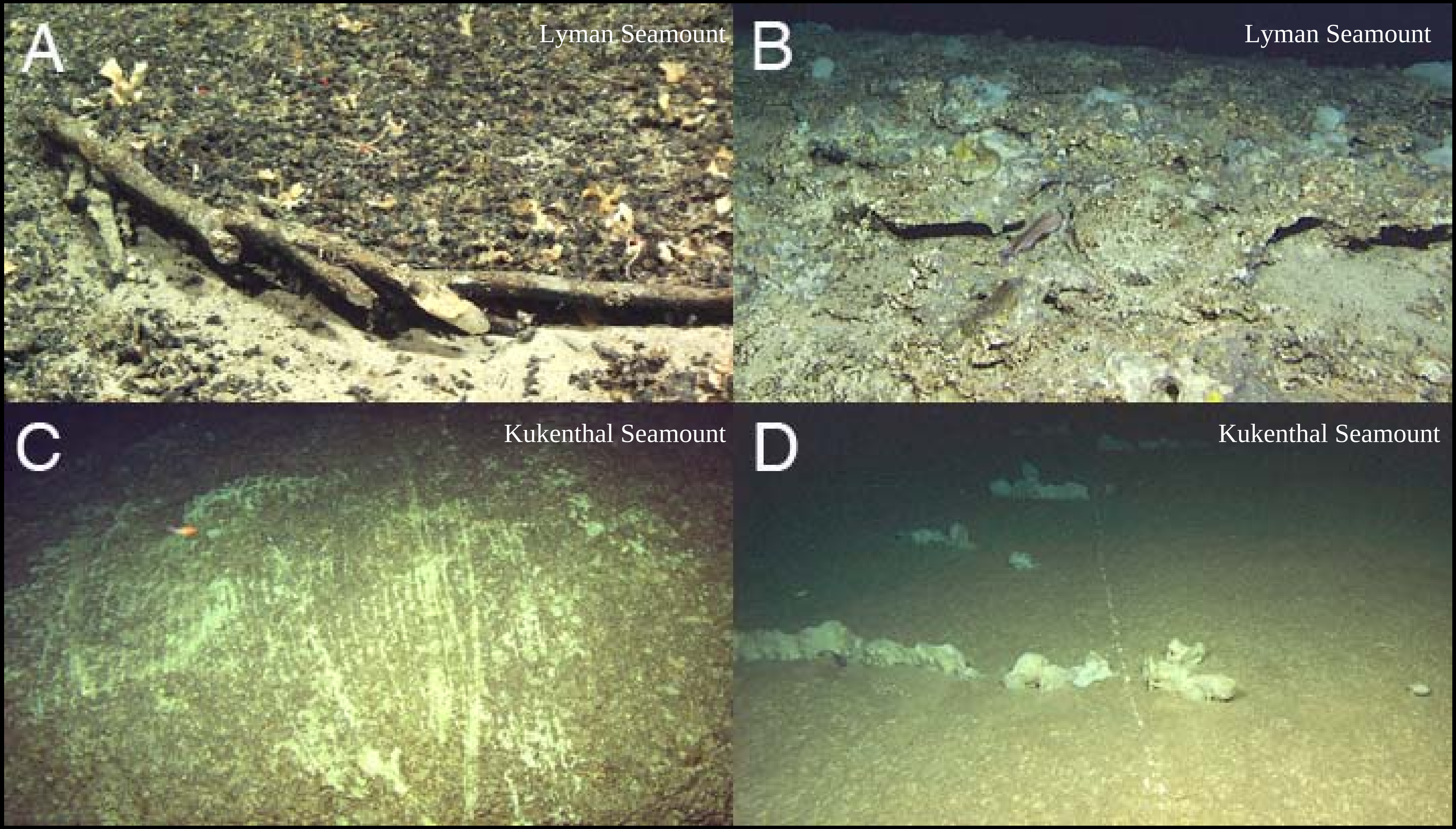
octocoral *Metallogorgia* & brittle star *Asteroschema*

octocoral *Candidella* & brittle star *Ophioplinthaca* & scaleworm *Gorgoniapolynoe*

brittle star *Ophioplinthaca* & *L. pertusa* & *Desmo-phyllum dianthus*



Predicted flow (blue arrows) of the Gulf Stream at 3000m depth (mean flow in solid red) across the New England Seamounts. Modified from Houghton et al., 1977; Olu, 1994.



"Fisheries-related habitat damage" on the Corner Rise Seamounts (North Atlantic)

(A) broken *Paragorgia* branches (~40 cm long) (1450m depth);
(B) broken ground (mainly *Lophelia pertusa* rubble) resulting from heavy trawl gear on Lyman Seamount (1427m depth);
(C) parallel scour marks generated by chain-linked trawl nets on the edge of the summit plateau of Kukenthal Seamount (769m depth); and
(D) a single drag mark, perhaps from a metal weight suspended from the trawl footrope, that has cut through sponge communities (750m depth).

All pictures taken with ROV Hercules (DASS05_NOAA_IFE). (Waller et al., in prep)

Potential Impacts of Exploration and Mining

Exploration (mapping, imaging, precision/limited sampling, testing of recovery systems; evaluation of environmental, technical, economic, commercial factors):

Potential Positive Impacts:

- Discovery of new species, adaptations, evolutionary pathways
- Environmental and biological assessments of targeted seamount(s)
- Constraints on models of important integrated processes associated with seamounts

Exploitation (commercial recovery of cobalt crust):

Potential Negative Impacts:

- loss of habitat, prohibiting connectivity and reducing genetic diversity
- degradation of habitat quality
- local, regional, or global extinction of endemic or rare taxa
- decreased diversity (all levels: genetic, species, phylogenetic, habitat)

What Do We Need?

Want to know who is there?

• ¶ Tandem molecular and morphological systematic approaches hold the greatest promise for developing classification schemes that reflect evolutionary relationships that provide a framework for understanding the processes creating and maintaining biodiversity

Want to know if and how seamount populations among Seamounts (chains or “distant” guyots) are connected?

• Measure physical oceanographic flows, habitat availability, rates of colonization, growth, and dispersal in concert with genetic data to understand mechanisms of connectivity and evolution

What Do We Need?

Want to know what's maintaining biogeographic boundaries and species' migration patterns?

- combination of systematic and phylogenetic assessment among taxa is a prerequisite for understanding biogeographic patterns and the dispersion of species over evolutionary time (e.g., across ocean basins or between Smt chains)

Want to know the affects of mining on seamount populations?

- PRE-disturbance genetic characterization of connectivity to neighboring seamounts/habitats- develop conservation targets

- POST-disturbance genetic characterization of connectivity to neighboring seamounts/habitats

What Do We Need?

• to realize we can not extrapolate dispersal/connectivity results of any taxa or seamount to another. For example, from the South Pacific to the Equatorial Pacific

• to realize that species characterized as having “limited larval dispersal” can travel large oceanic distances (e.g., deep-water clams)

• to realize what an “isolated seamounts” is- can define as “isolated” if the recent exchange of genetic information is not statisticallly significant (distance $\neq$ isolation)

• to realize that any plan to assess seamount diversity, endemism, and connectivity should include the integrated use of genetic approaches

USS San Francisco

crashed into 2-km tall uncharted Seamount

- Los Angeles class nuclear submarine ran aground in route from Guam to Brisbane, Australia - 8 January, 2005
- One sailor killed, 115 injured
- Crash depth ~160 m, speed 33 kn, Sonar measured a depth of 2000 m 4 minutes before crash
- 30-hour trip back to Guam, crew managed to keep the sub from sinking

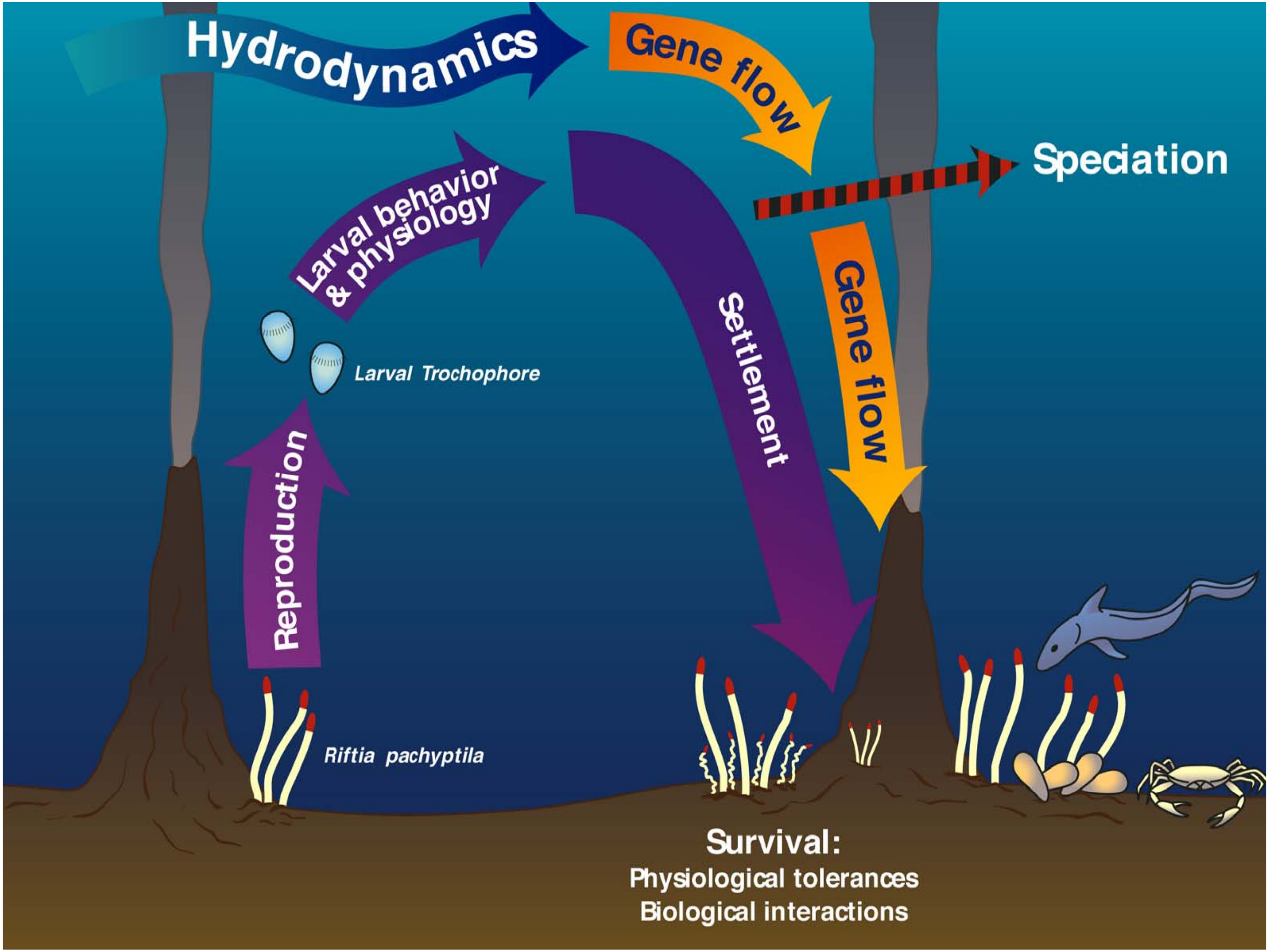
[redacted]

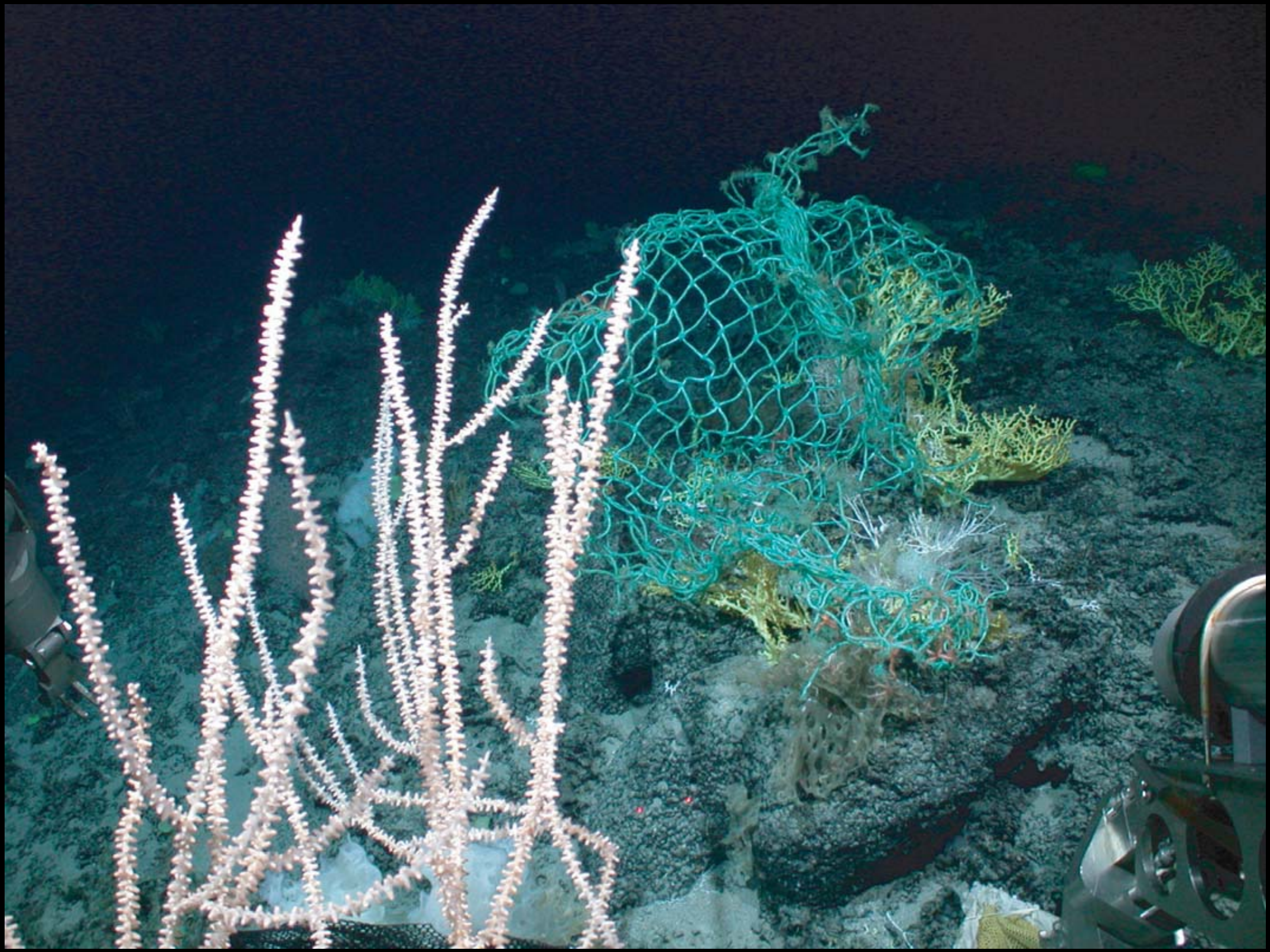
Workshop Goal: to ascertain the potential impact of exploration and mining of deep-sea cobalt-rich ferromanganese crusts on seamount fauna

- assess patterns of diversity and endemism on seamounts and factors that drive these patterns

- identify gaps in current knowledge (encourage collaboration in these areas)

- provide suggestions and recommendations to assist the development of environmental guidelines for the protection of seamount fauna

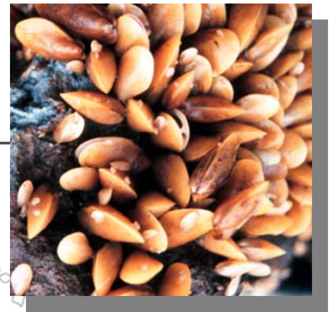

Hydrodynamics
Gene flow
Speciation
Larval behavior & physiology
Settlement
Gene flow
Larval Trochophore
Reproduction
Riftia pachyptila
Survival:
Physiological tolerances
Biological interactions

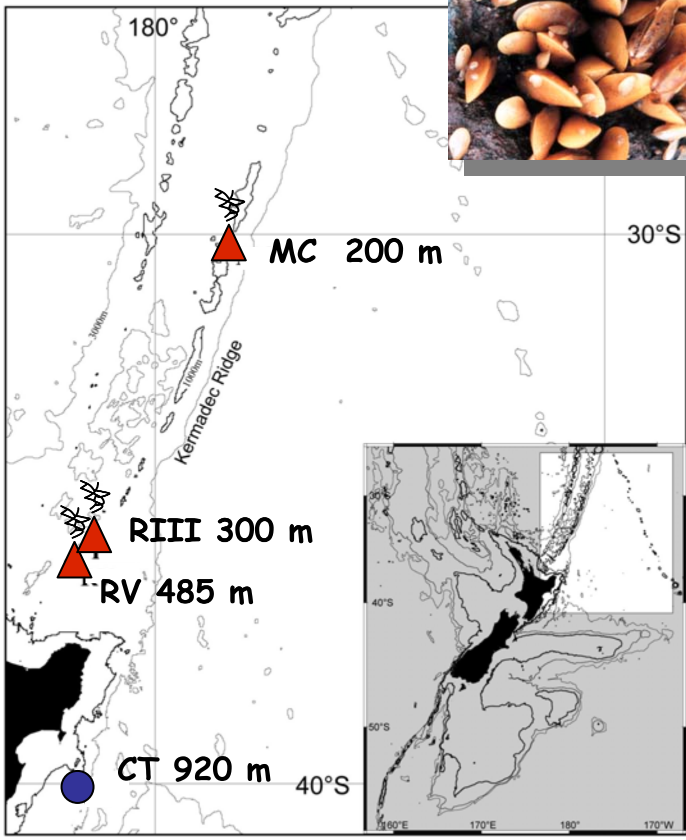


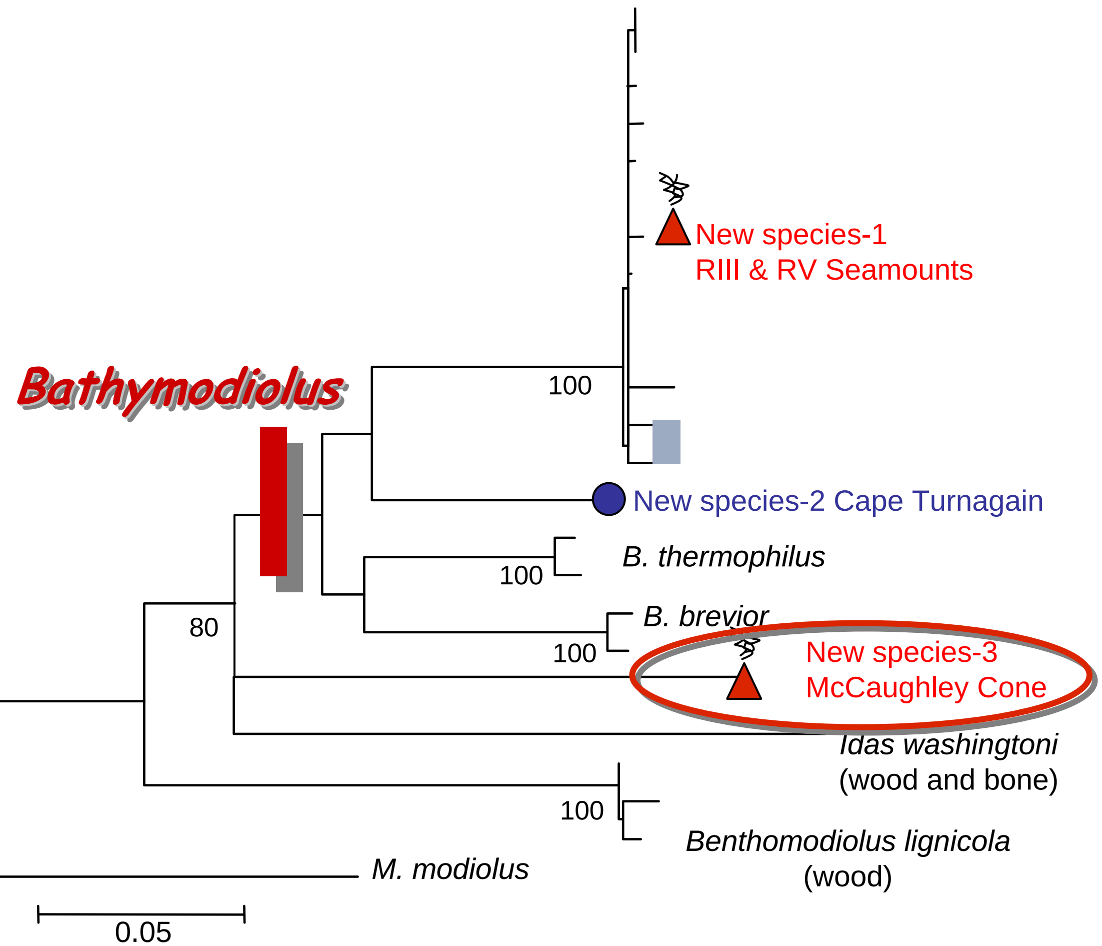
Muir Seamount

Molecular Phylogenetics -evolutionary relationships

New Zealand species of hydrothermal vent mussels

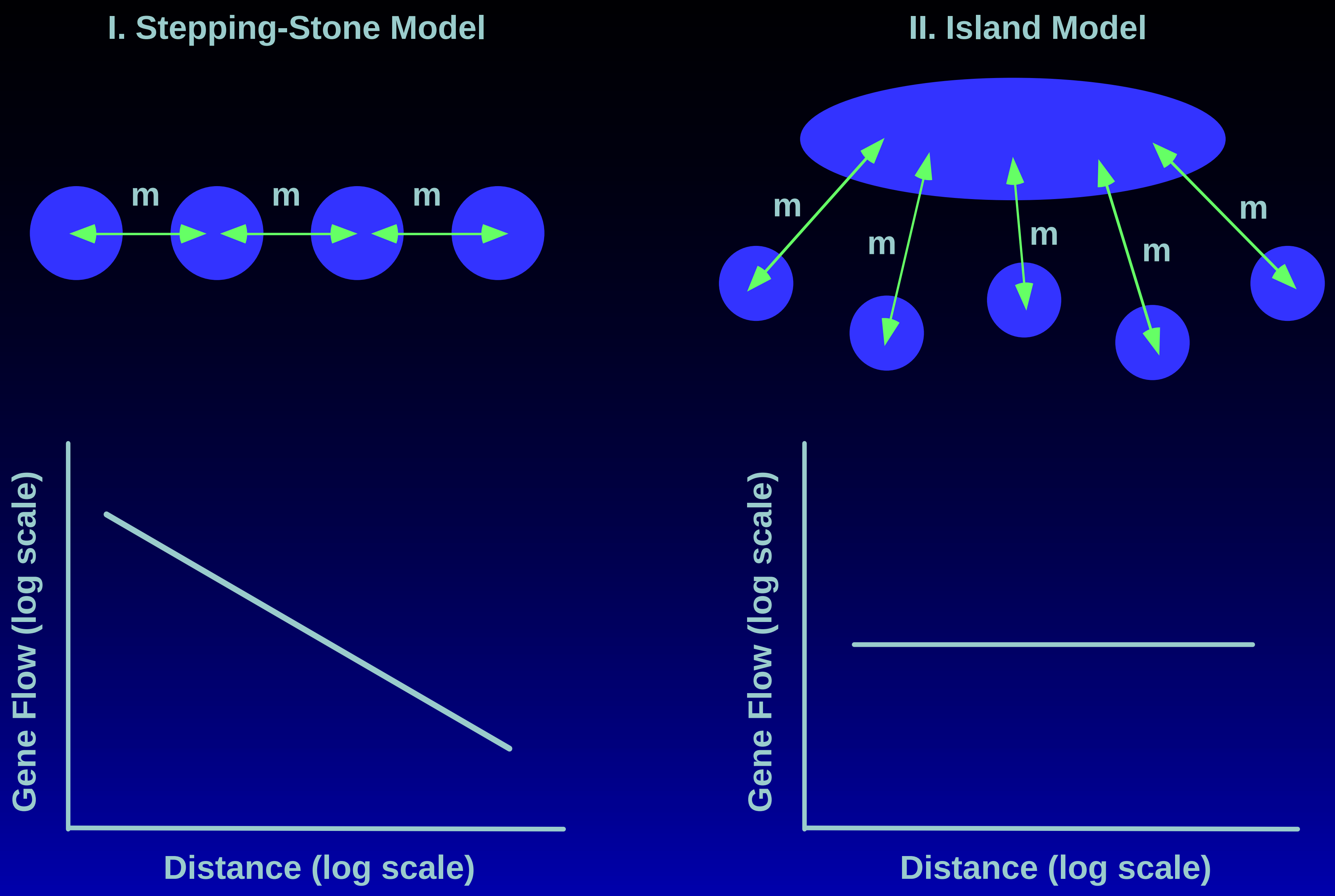






Smith PJ, McVeagh SM, Won YJ, Vrijenhoek RC (2003) Genetic heterogeneity among New Zealand species of hydrothermal vent mussels (Mytilidae: *Bathymodiolus*). Marine Biology, in press

Gene Flow Models



Characteristics of seamount settings important to biology

- chemoautotrophic production present, but lower rates of primary productivity

- no flux of hydrothermal fluids, but sulfides may cap thermal systems and begin to flow once disturbed by mining activities (ODP experiences)

- with macrofaunal invertebrates, possibly some (or many) that are endemic, but much lower biomass; no known examples of symbioses

- habitat duration longer than that of active vents

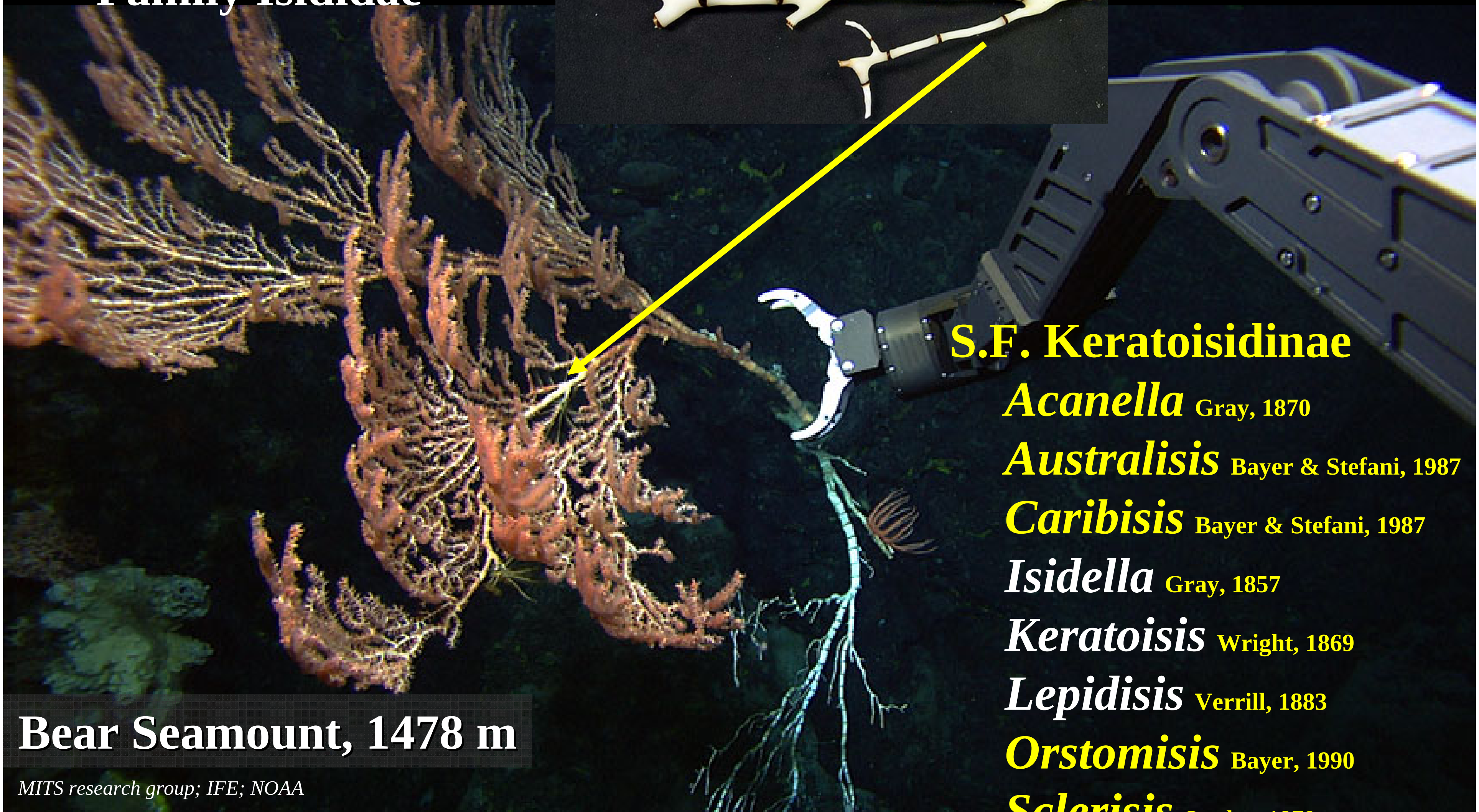
- degree of isolation similar to that of active vents

- reproduction – relevant conditions different (e.g. habitat longevity, population density, food availability), so potential for different adaptations

- larval transport – plume-generated currents absent

“Bamboo corals”

Family Isididae



S.F. Keratoisidinae

- ***Acanella*** **Gray, 1870**
- ***Australisis*** **Bayer & Stefani, 1987**
- ***Caribisis*** **Bayer & Stefani, 1987**
- ***Isidella*** **Gray, 1857**
- ***Keratoisis*** **Wright, 1869**
- ***Lepidisis*** **Verrill, 1883**
- ***Orstomisis*** **Bayer, 1990**
- ***Sclerisis*** **Studer, 1879**

3 additional subfamilies

S. France, unpub. data

***Lepidisis*:**

…simple *or branched*, the branches, when present, arising from the horny joints (Verrill 1883)

…*branched* from the nodes or the internodes… (Grant 1976)

"*Unbranched* Keratoisidinae " (Muzik 1978)

MITS research group; IFE; NOAA

MITS research group; IFE; NOAA

***Keratoisis*:**

…branch from the internodes, *or are unbranched*… (Grant 1976)

Kükenthal, W. 1919. Gorgonaria. Wissenschaftliche Ergebnisse Tiefsee Expeditionen auf dem Dampfer "Vadivia" 1898-1899. 13(2): 1-946.
Grant, R. 1976. The marine fauna of New Zealand: Isididae (Octocorallia: Gorgonacea) from New Zealand and the Antarctic. New Zealand Oceanographic Memoir 66: 1-56.

Potential impacts of exploration and mining

“There is no imminent threat to the entire global vent fauna from mining or any other human activity.”

“Concentration of mining activities in areas such as the southwest Pacific back arc spreading centers will, however, produce local and even regional effects on vent organism abundance, to the point where the survival of some species could become an issue.”

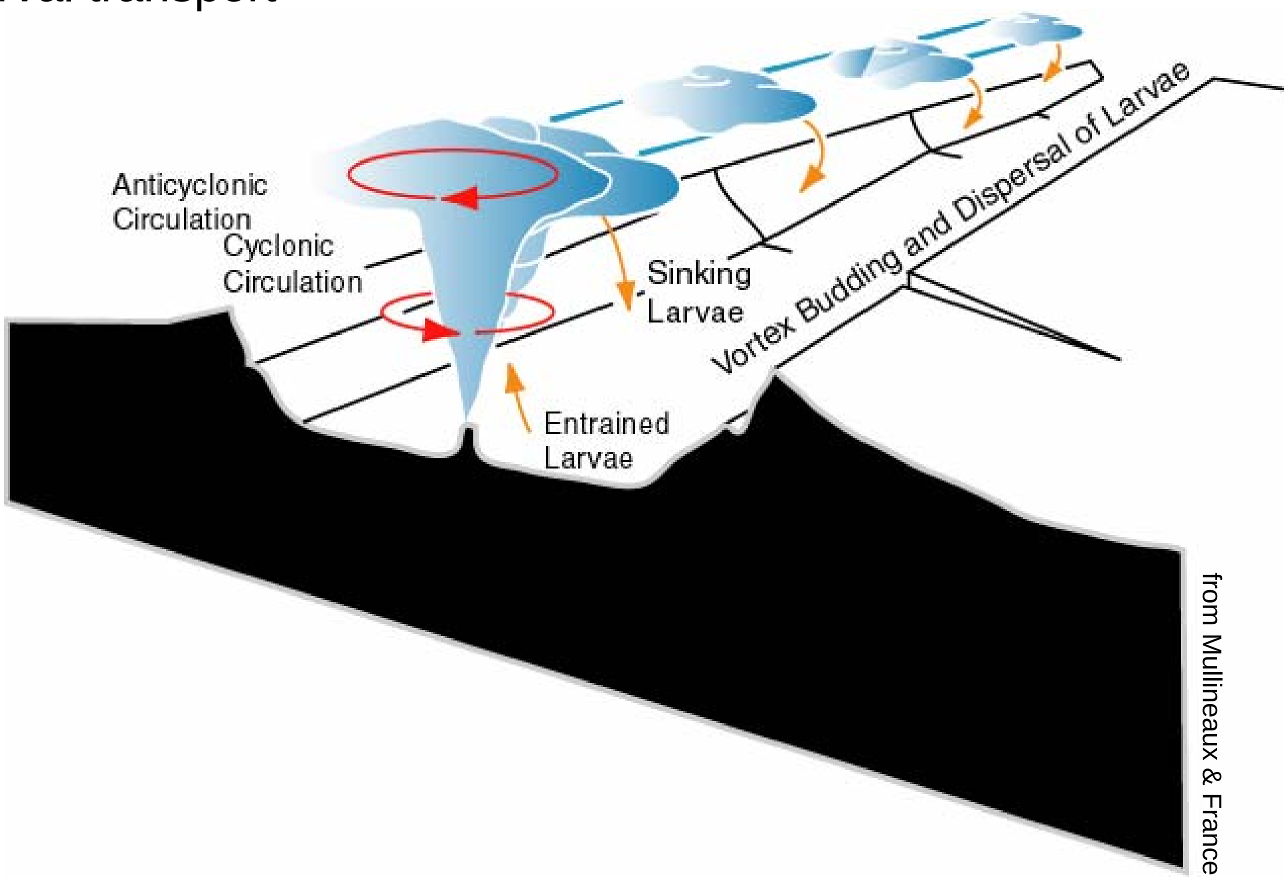
Juniper, K. 2002. Polymetallic massive sulphides and cobalt-rich ferromanganese crusts: status and prospects. International Seabed Authority, Jamaica.

“The anticipated consequences of sulphide mining will be the release of toxic elements and, more importantly, of fine particles.”

Scott, S. 2004. Abstract. Workshop for the establishment of environmental baselines at deep seafloor cobalt-rich crusts and deep seabed polymetallic sulphide mine sites in the Area for the purpose of evaluating the likely effects of exploration and exploitation on the marine environment. Kingston, Jamaica

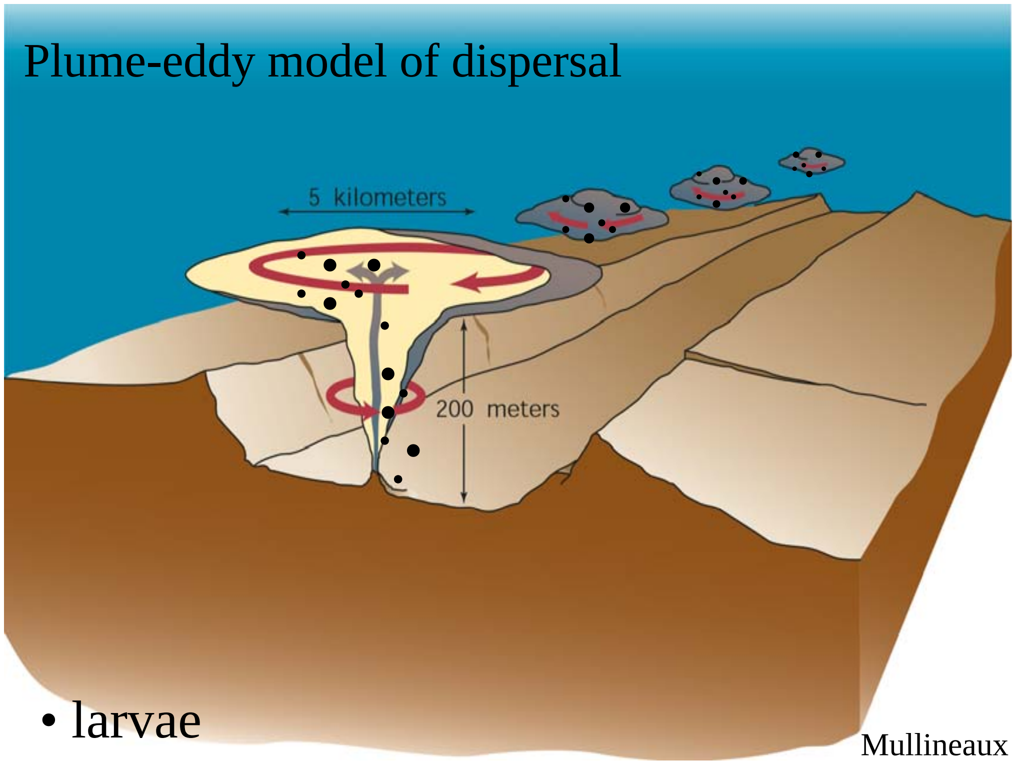
Characteristics of active hydrothermal settings important to biology

Larval transport



from Mullineaux & France

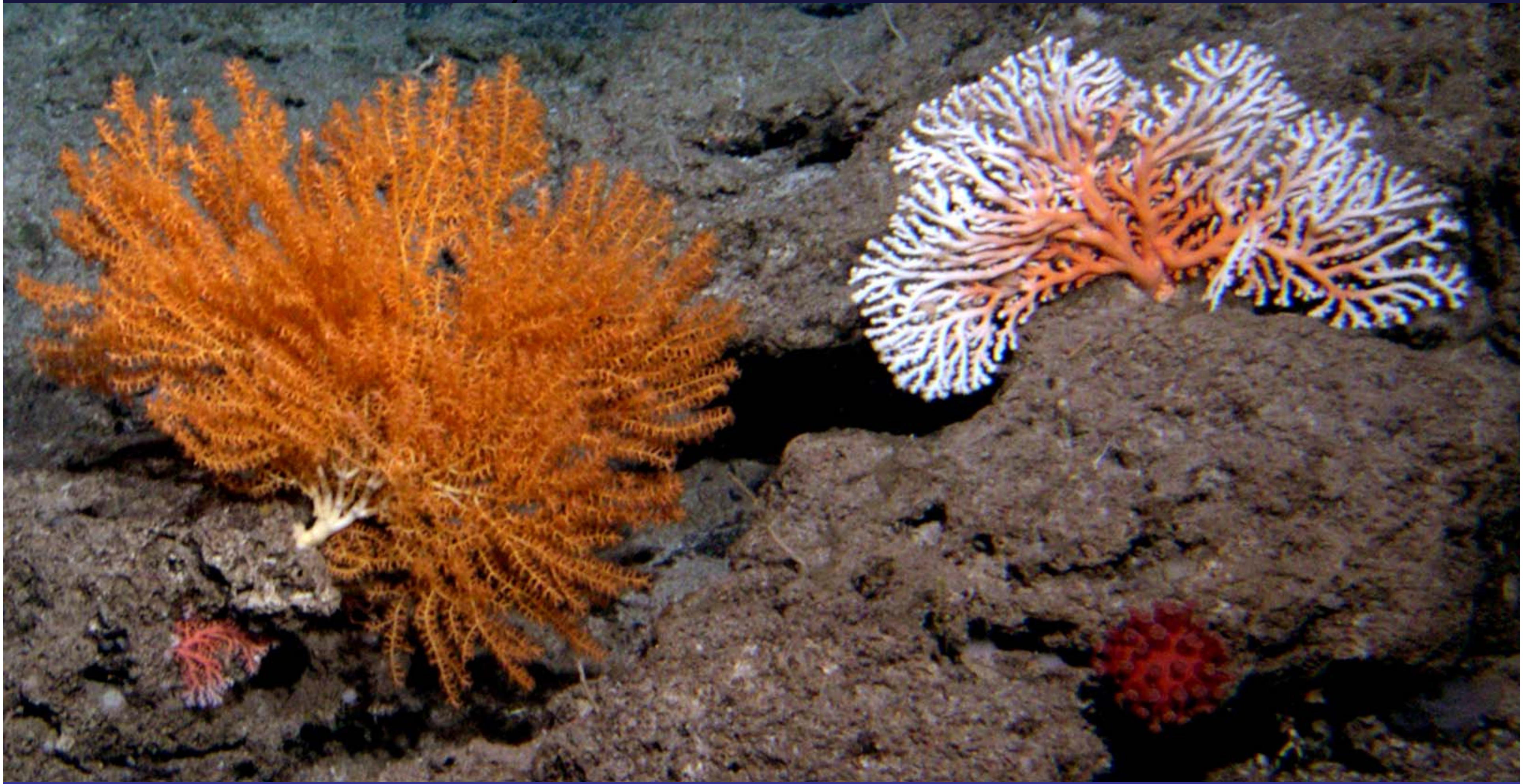
Mechanisms of Dispersal



Riftia free-swimming trocophore larvae - 40-day lifespan *plus* current meter data suggest larval retention (~100km) within ridge segments on the East Pacific Rise.

(Marsh et al. 2002)

Population Genetic Structure of the Hawaiian Precious Coral, *Corallium secundum*, Based on Microsatellites



Amy Baco

Data required to establish environmental baselines in exploration areas

Standard criteria to be used in environmental assessment

- Characterization of the type of disturbance
- Estimation of the percent loss of [seafloor vent] habitats
- Identification of affected seafloor organisms
- Dose-response characteristics of plume fallout

Baseline information required

- Distribution of habitat within the potentially affected area
    - Species composition and community structure
    - Basic biology of species

Data required to establish environmental baselines in exploration areas

Key data, activities include:

- Detailed maps of the size and distribution of target sulfide deposits as well as of critical or special habitats such as non-target seamounts and the zone likely to be influenced by particle deposition.

- Measurement of the prevailing hydrographic regime and development of particulate fallout models to predict the suspended particulate and depositional gradients generated by mining activity, where critical habitats occur in the exploration area. This should include an assessment of the depositional shadow of specific minerals/metals potentially toxic to marine organisms (e.g., copper).

- Determination of the extent to which the biota of the region is endemic or cosmopolitan (using genetic data).

- Description of habitat through detailed imaging surveys
  - Determination of "intimacy" of host - associate (fauna can't colonize seamounts if ho
- Evolution of populations can provide evidence for sources and sink populations - dispersion of species over seamounts.
- Modelling larve dispersal through physical oceanographic modeling

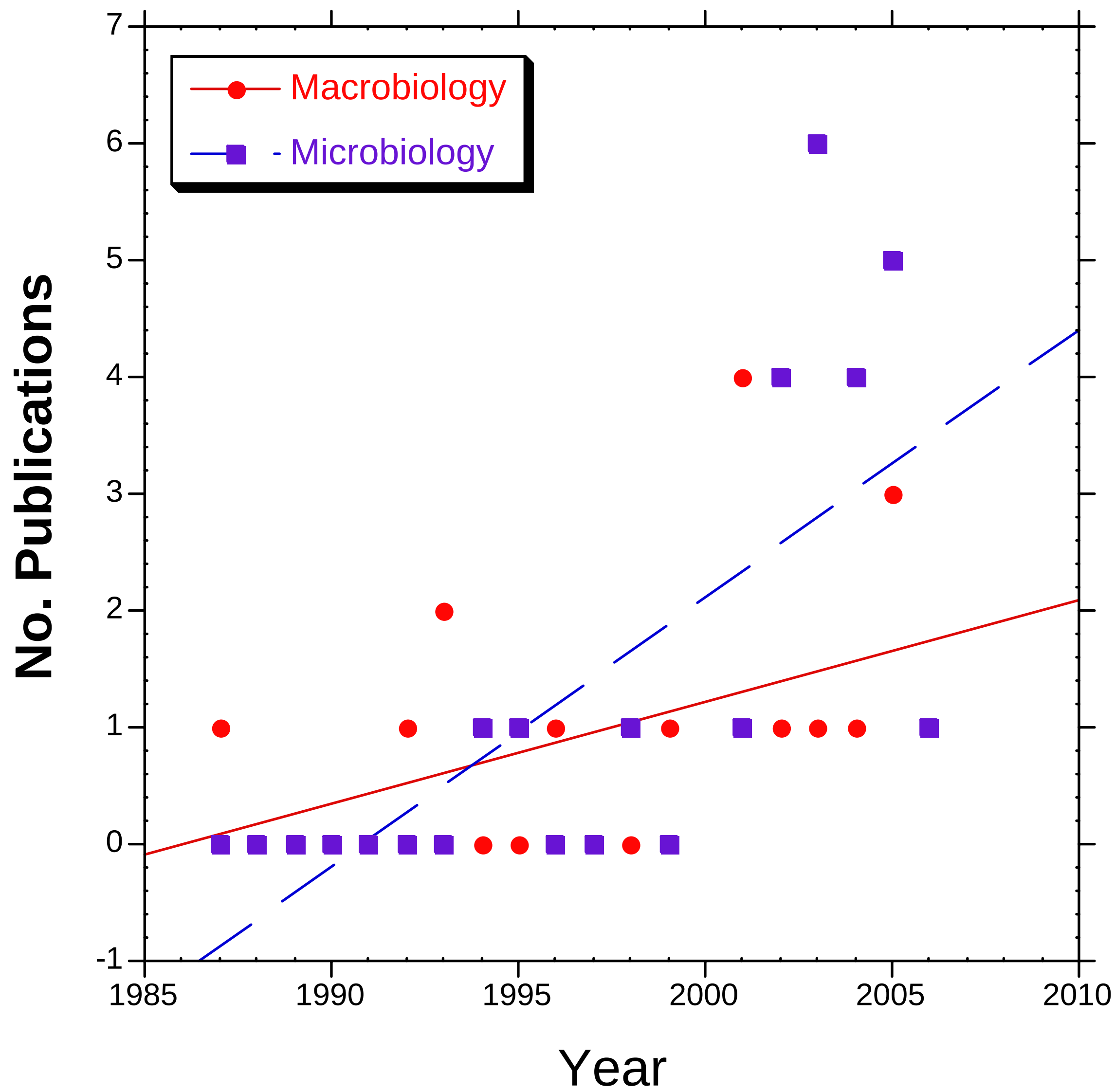
Data required to establish environmental baselines in exploration areas

Types of baseline data that will assist in assessment of impact of mining activities:

- Microbial diversity, biomass, primary productivity, biogeochemistry
- Taxonomic composition, species richness, diversity, evenness, biomass, abundance of
  - “strategic” sulfide-associated mega-, macro-, meiofaunal communities
  - background communities in areas within the depositional shadow
  - reference communities outside depositional shadow
- Genetic diversity of strategic species of background and sulfide habitats
- Trophic relationships among vertebrates, invertebrates, microbes, and mineral deposits
- Species ranges (can they be found outside the area likely to be impacted by mining) and degree of endemism of microbial, invertebrate taxa in association with sulfide deposits
- Identification of adaptations specialized for polymetallic mineral deposits
- Dose-response parameters especially for background fauna or fauna of critical habitats within the depositional shadow

Summary of Some Key Thoughts and Observations

- the connectivity of seamount fauna among seamounts is essentially unknown, but knowable
- fauna associated with seamounts are potentially of tremendous importance to biotechnology, remediation, and understanding the metabolic menus exploited by life on Earth and potentially elsewhere
- comparison of genetic information among seamount fauna can tell us the systematic (taxonomic) status and evolutionary relationships
  -important given the current problem dearth of taxonomists to undertake morphological descriptions of species
- baseline biological assessment of seamounts must consider whether neighboring non-mined habitats host same species and connected populations (the key to gene flow is the dispersal of larvae and available habitat)
- importance of reserves or sanctuaries as required in the ISA Draft Regulations cannot be underestimated, especially given our lack of knowledge of the biology of inactive seamounts– but these may not be easy to identify and require careful consideration and definition as to what is acceptable in a given region



Macrobiology
Microbiology
No. Publications
Year
7
6
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4
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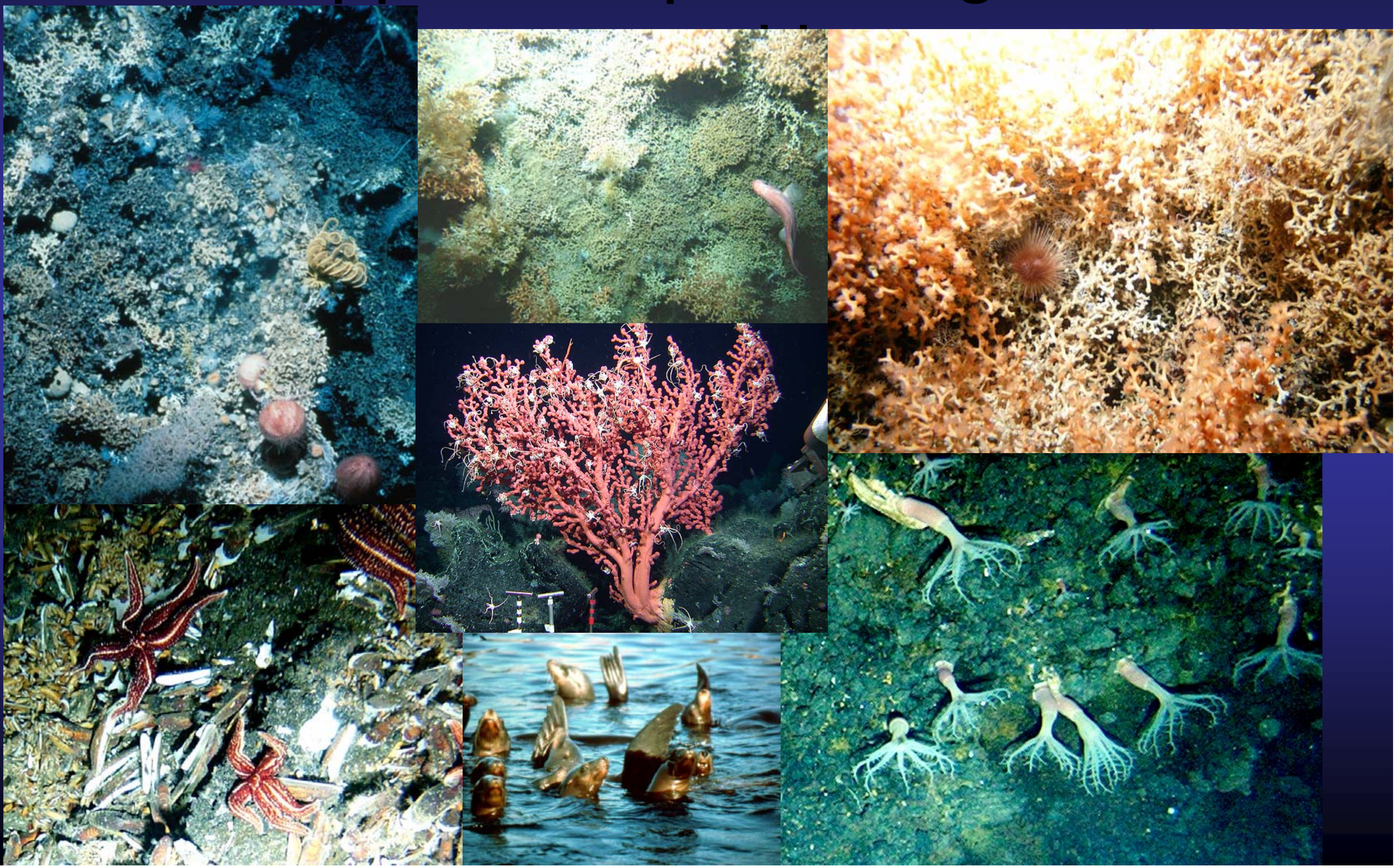
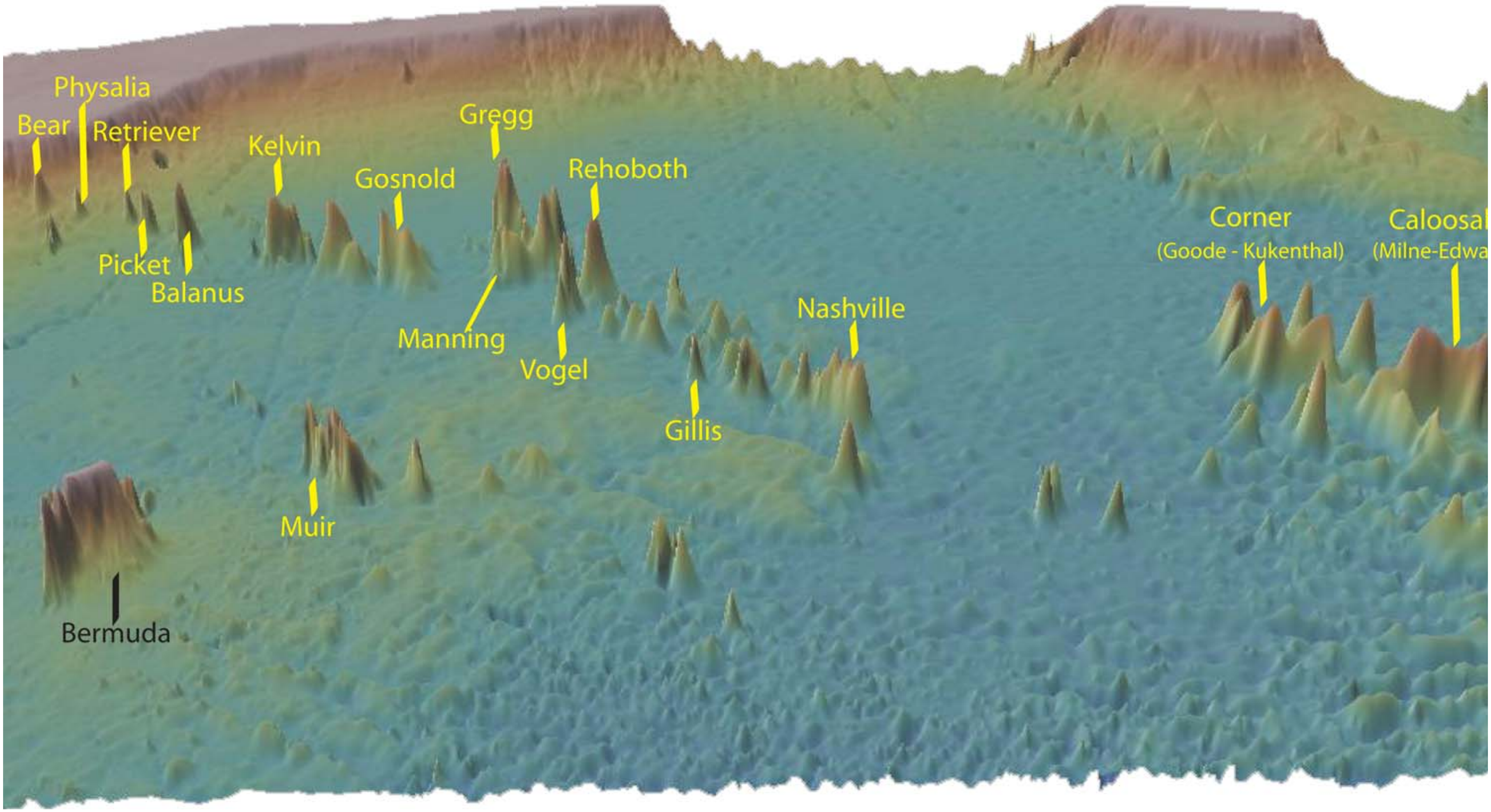
1985
1990
1995
2000
2005
2010

seamounts??

Support unique biological




Physalia
Bear
Retriever
Kelvin
Gregg
Gosnold
Rehoboth
Picket
Balanus
Corner
(Goode - Kukenthal)
Caloosal
(Milne-Edwa
Nashville
Manning
Vogel
Gillis
Muir
Bermuda