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Remote Species Identification

Workshop Report
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Bryan Coles at Raytheon Systems Company provided the cover photograph - a pseudo-color image of a coral reef in the Dry Tortuga Island obtained with a multi-channel laser line scan system that was designed and built by Raytheon Systems Company. The raw data were processed by Andy Nevins of the Naval Underwater Warfare Center/Coastal Systems Station. The data were obtained in 1996 as part of the Coastal Benthic Optical Properties (CoBOP) Program under the sponsorship of Dr. Steve Ackelson of the Office of Naval Research. The image is used with permission.

Jesse Ausubel and the Alfred P. Sloan Foundation provided funding for the workshop and report production.

Overview

INTRODUCTION TO CENSUS OF THE FISHES
Ausubel

Jesse Ausubel of the Alfred P. Sloan Foundation gave a general introduction to the ongoing exploration of the Census of the Fishes (COF) concept and the Foundation’s role in the evolving program. COF can be thought of as a marine counterpart to a previous program the Foundation has been instrumental in organizing - the Digital Sky Survey. Rather than look beyond our environment, COF seeks to explore the oceanic realm, perhaps by mapping the biota of the world's oceans. For the past two years, the Foundation has been exploring the limits and limitations of COF, as regards the following three questions:

1. Is COF worth doing?
2. Is the technology feasible at a reasonable cost?
3. Do the current stakeholders/science community want COF to happen?

To answer these questions and shape the program, the Foundation has sponsored a range of workshops aimed at addressing a particular aspect of COF:

- Diversity of Fishes
- Non-Fish Nekton
- Benthic Environment
- Technology
- Tagging

as well as two more general meetings aimed at developing the COF concept. This workshop is an exploration of a specific task within COF - cataloging the marine species of the world.

INTRODUCTION TO REMOTE SPECIES IDENTIFICATION
Parrish

COF is both an exploration of biomass and biodiversity. This workshop concentrates on the latter category by exploring potential technologies which could be used, separately or in concert, to detect and identify marine species without capturing the organism. Why is species identification important? First, because we can relate it to things known, organism identification facilitates knowing how it works (in both a physiological and an ecological sense). Second, species identification gives us a window into the evolutionary history of a taxonomic group and insight into the evolutionary ecology of the ecosystem of which it is a part. Third, if mapped, (e.g., species distribution) species identification allows us to track introductions and extinctions, the currency of biodiversity change/loss. Fourth, if quantified, (e.g., species' abundance) identification allows us to track biomass-biodiversity relationships which change as a result of both natural and anthropogenic forces. With regard to marine systems, developing remote species identification (RSID) capabilities is useful because there are large tracts of ocean which are inadequately sampled, either because it is technically too difficult and/or because the vastness of the ocean makes comprehensive sampling financially impossible.

Experts from four different technological bases (acoustics, optics, chemical ecology, molecular biology) were assembled at this workshop to review the available technologies within their field (Day 1 task) and to brainstorm possible designs of a multisensor system - the Super-Predator - which will engage in RSID (Day 2 task). Prior to this workshop, four participants were asked to "seed" the discussion by writing comprehensive overviews of the technologies they saw as appropriate to RSID:

Optics - James Bohnsack
Acoustics - John Horne
Chemicals - Dick Zimmer
Molecular - Ann Bucklin

All participants recieved a white paper packet, which faciliated two goals: First, within discipline, participants had the opportunity to modify and build on the white paper presentations. All four white papers, modified by discussions on Day 1, are included in an appendix to this report. Second, among disciplines, participants had the opportunity to "learn the basics." This facilitated informed discussions and brainstorming, especially during the Super-Predator invention task.

SUPER-PREDATOR CONCEPT

Presumably all organisms have the ability to distinguish conspecifics from other species. Many species are able to detect functional classes of organisms - competitors, predators, etc. - to which a specific behavioral response is appropriate. However, higher order generalist predators appear to have the ability to detect and identify a range of other species, namely their prey. In fact, predators must continually make a series of foraging decisions along the continuum from detection to consumption:

1. Is there something there?
2. Is it prey?
3. What kind of prey is it, or, How does it rank in the hierarchy of preferred prey types?

The Super-Predator concept springs from this species identification ability of biological predators. Instead of using a variety of manmade sensors independently to assess and catalog the environment and its biological contents, perhaps we can take a lesson from biology and manufacture an integrated sensing system modeled after organisms which depend on species identification for survival.

Super-Predators are machines or networks of machines which remotely detect and identify biological organisms using a variety of modular sensors in an integrated sensory system. Rather than capture and consume their prey, Super-Predators capture information about species identity, as well as a range of ancillary information which might include distribution and abundance; size, age, and health; individual identity; and environmental quality.

Predator-SuperPredator Analogies

Sensory Integration

Predators use a range of sensory systems to detect and identify prey in a noisy environment (Gr nbaum 1997). All vertebrate predators possess passive acoustic abilities (i.e., hearing) and some species use active acoustics to detect prey (i.e., cetaceans). Sharks have been able to locate prey from distances of up to 250m by orienting towards irratically pulsed low frequency (below

10 Hz) sounds produced by swimming injured fish (Myrberg & Nelson 1991). Most predators use optics (i.e., vision) to detect, track, and identify prey at slightly closer ranges, usually confined to a forward cone of vision which may stretch to 60-70m in the clearest of oceanic water. Many predators have chemosensory abilities (i.e., smell) used to detect prey exudates. In addition, many organisms have species-specific chemical cues, or pheromones, to which conspecifics are exquisitely sensitive. Pheromones are used for mating, as well as for aggregation, alarm, and group cohesiveness (Atema 1979, Pfeiffer 1982, Hara 1993). Short-range chemosensing (i.e., taste) is one of the last senses in the detection-to-consumption continuum. Each sensory system has a working range and resolution which depends on the internal sensory reception structure as well as the physics of the environment. All systems are integrated, giving the predator the ability to groundtruth detections/identifications internally.

Biological predators have a subset of all known sensory systems and a subset of sensors over the possible working range of each sensory modality. Super-Predators have all sensory modalities (i.e., acoustics, optics, chemosense, electromagnetic), usable over the range of the sensor(s). In addition, Super-Predators may possess sensory modalities not currently available in the biological world (e.g., DNA fingerprinting) and/or be able to change the local physical environment to increase sensor abilities (e.g., light a dark environment).

Environmental Signature

Predator-prey interactions have been described as an evolutionary arms race (Dawkins & Krebs 1979, Feder & Lauder 1986). Predators rely on an integrated set of sensors and locomotors to detect, pursue, and capture prey, while prey depend on an equally sophisticated sensory system to escape. One way predators "win" is by evading the detection abilities of the prey until the chance of prey capture is high. Thus, predators must be stealthy. Similarly, Super-Predators are silent interlopers in the environment (e.g., minimal noise, minimal light, etc.) such that they may either approach organisms without reaction and/or become behaviorally invisible to the surrounding ecosystem.

Capture Strategies

Predators employ a detection-to-capture strategy, to which their sensory systems and morphology (e.g., locomotory structures) are adapted. Four basic predator strategies include: *Sit-and-Wait* predators are relatively immobile, and may be cryptic, allowing the prey to approach before striking (e.g., grouper). *Ambush* predators are a variant of sit-and-wait in which the predator attracts the prey before striking (e.g., anglerfish). *Stalking* predators sneak up on prey, minimizing the pursuit distance before initiating an attack(e.g., Pike). *Pursuit* predators chase their prey down (e.g., tuna). Super-Predators emulate predators in that platform deployment mimicks strategy: Moored Super-Predators sit-and-wait. Moored Super-Predators with attractants ambush organisms. Drogue Super-Predators stalk organisms moving within the same water body. ROV and AUV Super-Predators may stalk or actively pursue organisms, depending on the disparity in platform to organism size. In addition, drogue, ROV, or AUV deployed Super-Predators may contain attractants to locally concentrate organisms before moving.

Prey Size

Biological predators concentrate on a discrete size range of prey, usually based on gape or appendange limitations (Kislalioglu & Gibson 1976). Within the range of physically available prey, even generalist predators may actively choose between alternate prey types, pursuing some over others. Super-Predators have a virus-to-whale capability. Alternatively, they may be designed to concentrate on a specific size range of organisms, without preference within size class. Super-Predators may also be designed to detect and identify specific taxa as a function of their ability to selectively attract and concentrate taxon members to within identification range.

DAY 1

Day 1 Agenda

9-10 am	Introduction	Pacific Forum
10-12 am	Breakouts Optics Acoustics Chemical/Molecular	Conference Room Pacific Forum 2nd flr. Break Room
12-1 pm	Lunch	
1-4:30 pm	Breakouts Optics Acoustics Chemical/Molecular	Conference Room Pacific Forum 2nd flr. Break Room
4:30-5:30 pm	Plenary/Breakout Summation	Pacific Forum
7 pm	Dinner	

DAY 1 GROUP TASKS

Workshop participants were divided into three groups along disciplinary lines: Optics, Acoustics, and Chemical & Molecular. Using the white papers (Appendix) as a starting point, each group was tasked with constructing a matrix of available and on-the-horizon technologies which might be used to address remote species identification. Using the Super-Predator metaphor, how would the predator work if it could *only* use technologies within discipline (e.g., only optical or only chemical). The matrix contained the following categories:

- Technology - name and brief description of the technology (e.g., low frequency sonar; HPLC)
- Organism - primary, secondary, etc. taxa sampled (e.g., zooplankton; fish). This category may also be size-delineated (e.g., zooplankton size; whale size)
- Habitat - primary, secondary, etc. habitat sampled (e.g., benthos; open ocean)
- Platform (e.g., ship; mooring; submersible)
- Cost - in round numbers (can also be unknown)
- Availability - now, soon, later (if latter categories, estimate years to on line)

As added conceptual constraints, groups were asked to consider the following issues:

- What is a species? Should we use another moniker more relevant to size-shape? Alternatively, for technologies such as molecular techniques should we reduce our frame of reference further to populations, subpopulations, and even individuals?
- The problem of identifying new organisms versus correctly identifying known ones. How does remote technology quantify new?
- The definition of remote. Remote may be not-in-hand, as in remote sensing. Alternatively, remote may be non-invasive, as in collection of shed scales or exudates. Finally, remote might be a biopsy collected and returned to the scientist for latter identification.
- Passive versus active (i.e., attraction) sampling. Should sampling interfere with the diversity and distribution of organisms sampled?
- Data storage, retrieval, and processing.
- Space-time sampling scale.
- Navigation. How does the technology "know" where it is?
- Other types of data inadvertently collected, which may be useful to science/resource management.
- Biosensors. Rather than create machines from scratch, can we assemble bio-machines which couple biological sensors with amplified recorders?

DAY 1 GROUP ASSIGNMENTS

Optics	**Chemistry/Molecular**	**Acoustics**	**Floating**
John Ohnsack, *Rapporteur* Bryan Coles, *Chair* Jim Harvey Percy Donaghay Edie Widder John Lindsay	Ann Bucklin, *Chair, Rapporteur* Dannis Hedgecock Dick Zimmer Peter Sorenson David Dixon	Rick Love, *Rapporteur* Peter Wiebe, *Chair* Manell Zakharia John Horne Carla Scalabrin Ole Misund Tommy Dickey Dave Mellinger Yvan Simard	Julia Parrish Jesse Ausubel

DAY 1 REPORTS

Acoustics Breakout Group
Rapporteur: R. Love

SYNOPSIS OF INITIAL EXPLORATORY CONVERSATION

Wiebe: Need to do biogeography of the oceans over again.

Zakharia: Acoustics can only identify animals of different shape, etc.

Parrish: Habits, behavior can help.

Horne: Resolution is an issue.

Misund: Groundtruthing is an issue - the need for direct sampling.

Wiebe: Single frequency sonars can not identify without groundtruthing.

Simard: Acoustics is best for biogeography not so good for biodiversity. Must employ statistics.

Wiebe: Complete biogeography - species and their abundance - needed.

Horne: Can we assume strong coupling between biology and physical oceanography? What about migratory species?

Love: Don't forget historical data.

Misund: Most of ocean is fairly unproductive.

Wiebe: Low production regions frequently have more species than high production areas.

Scalabrin: Acoustics is best for detection then use other systems.

Mellinger: Passive systems can be used to identify fish.

Zakharia: Passive systems best for cetaceans.

Simard: Would like rectangular beams.

Zakharia: Single ping versus multi-ping, echo analysis versus school analysis.

Simard: Canada developing survey design and species identification multi-channel acoustic system.

Wiebe: Black hole of bioacoustics - the material properties of the animals.

Horne: What about active control of properties by individuals?

SYNOPSIS OF BRIEF PRESENTATIONS OF VARIOUS ACOUSTIC SYSTEMS IN USE

Wiebe

Bio-Optical Multifrequency Acoustic and Physical Environment Recorder (BIOMAPER II)

- 40, 120, 200, 420, and 1000KHz on towed sled
- looks up and down
- video plankton recorder (VPR) and other bio-optical flowthrough sensors
- test cruise in July 1997

showed example where 40KHz seems to show something other than animals which show up at higher frequencies

__Zakharia__

- 20-80KHz, 100 elements, 50cm x 50cm, 6o beam
- low frequency: 20-40KHz, mid-frequency 40-60KHz, high frequency 60-80KHz
- 5 1KHz power amps
- chirps
- 7.5cm resolution
- tow speeds up to 10 knots
  pinged on schools of sardine, anchovy, and horse mackerel to train the neural network
- got echograms and individual pings
- used pulse compression
  after training, system identified known species correctly 67-75% of the time at sea on a single ping

__Love__

- TNT technique - creates broadband, low frequency "echo sounder," 0.5-25 KHz
- method determines swimbladder sizes/numbers
- need biological information to infer species from swimbladder sizes

__Mellinger__

- towed and fixed passive arrays
- can identify marine mammals, some fish, and some invertebrates. limited by noise and target proximity (fish - few meters; small cetaceans - few kilometers; whales - many kilometers)
- groundtruthing is a problem - do we hear them all or just some fraction (i.e., the ones making noise)

__Misund__

- integrated shipboard system
- use sonar for near-surface fish, echosounder for deeper fish, trawls for bottom fish
- acoustics are groundtruthed by trawling (e.g., to distinguish between herring and capelin)

ATTRIBUTES OF ACTIVE AND PASSIVE SYSTEMS

__Passive Systems__

- 20Hz - 30 kHz
- good for marine mammals, some fish, and a few invertebrates
- constrained by location, time, depth, reference sounds
- low frequencies use fixed arrays

- moored or drifting arrays could be used on "voyages of discovery"
- also towed vehicles and AUVs
- data storage, transmittal and processing are issues
- cost - $1-2K/channel, plus mooring, data storage, power, telemetry
- limited to marine mammals and spawning fish. could put arrays in select locations to assess such animals

Active Systems

Zooplankton

- 40 - 100 kHz
- can do body types (taxa) - not species
- can't get sizes now. Will we ever be able to?
- much equipment is available commercially
- include video and nets
- cost - $200-500K for multi-frequency towed system
- mooring Van Holliday's multi-frequency system costs about $60K

Fish

- 0.5 - 200 kHz
- some species can be identified under certain conditions
- any acoustic ID must be based on some previous information
- acoustics is good for biomass but not biodiversity
- fishery sonars are predominantly single frequency. we believe that multi-frequency systems will become more common.

CONCENSUS ON ULTIMATE ACOUSTICS SYSTEM

- multibeam (swath) - multifrequency
- frequency range - 1 to 1000 KHz
- calibratable
- integrated data streams (time, position, acoustics)
- constant beam angle across the frequency range
- "reasonable" size/weight
- compatible with passive array

Optics Breakout Group
Rapporteur: J. Bohnsack

SYNOPSIS OF PRESENTATION OF VARIOUS OPTICAL SYSTEMS

Widder

- bioluminescence is everywhere, an indicator of secondary production
- 75% of species and 95% of individuals are bioluminescent
- course scale: intensified cameras - dark of moon, fish stimulated
  - problem is moonlight
  - one sensor imaging
- fine scale: bathyphotometer - towed and moored - non-imaging
  - solid state - can't get gain
  - photomultiplier tube - PMT
  - HidexBP - high index definite bathyphotometer, now 44lt/sec operational
- micro scale: intensified camera on transparent 1m screen
  - organisms luminesce when they bump into screen - species specific label
  - time, space (e.g., nearest neighbor distances) and spectral properties
  - 50 micron dinoflagellate detectable at 1m
  - uses existing technology
- lux not a good measure alone
- low noise - high resolution - broadcast qualities
- gen - better resolution and signal to noise but poor resolution
- ben2 - intensification and amplification but lose resolution
- aerial systems for fish schools - lidar
- unmanned aerial vehicles

Harvey

- critter cams - optical installations on free-swimming marine mammals
- train animal to search for specific organisms (e.g., whales) as well as to return to pinger
- 400-500 dives/day

Lindsay

- geolocation - precision navigation a key issue - returning to the same spot to repeat surveys
- data integration with other surveys
- ultra shortbaseline acoustics
- visual range 1-2m
- monitor better than human visibility - 3x

Coles

- benthic mapping and species identification with fluorescence optics
- 2000 line resolution possible, 1000 standard
- at 1m2 - .25 inch resoltuion = 150 gbytes/m2
- 16 bit resolution dynamics, 2bytes/pixel
- dynamic range - 3 bands
- at 100 ft from the bottom, 140 ft field of view at 10 knots
- monochrome - 100-200 watts

- diversity in some cases good indicator of stress
- problem of inherent versus apparent signatures - need to remove optical properties of the water column
- merge acoustic and optical

Donaghay

- scale important
- microscale layers - need optical tools
    AC9 - nine channels
    spectrophotometer - 96 channels in real time
    absorption used to microsample a 2m wide band of red tide
- large scale
    herring in Puget Sound - keep in system, same layer
    discovered particulate material in the water column - organic structuring
    cue to sensors of organisms - spectral signatures and chemical signatures
- microturbulence using ruby laser
  2cm x 1cm - 10 micron resolution, 3 micron structure can be seen
- three-dimensional structure can be re-created - holography
    data processing is an issue
- combining techniques is important - acoustic, optical, physics, and discrete sampling

Chemical Signals and Molecular Techniques Breakout Group
Rapporteur: Ann Bucklin

SYNOPSIS OF INITIAL EXPLORATORY CONVERSATION

Chemical Signals

Chemical Taxonomies

defense compounds - doesn't have to be species-specific
sex pheromones/steroid metabolites
secondary metabolites - all water insoluble; better to use DNA

- want water soluble moieties
- insects - mating pheromones: unique blends but not unique components
- may be specific to one sex
- chemical catalogue
- now possible to answer number of genera
- some signature molecules (e.g., sea lamprey)
- ocean concentrations much lower than concentrations in spawning streams
- marine species less well known than freshwater species
- need physical context/transport system
- biosensors - olfactory neurons, artificial noses

Summary
There are a number of water-soluble moieties (including defense compounds and pheromones) that have specificities ranging from one individual, one sex, one species, one genus to one functional group. These chemical moieties may be useful for remote species identification, since they probably detectable at very low concentrations and may be relatively persistent in the aqueous marine environment. Thus, it might be possible to design and deploy remote detectors, to indicate that a member of a targeted species or group had been in the area within the past hours or days.

Implementation of these detection systems will require some research and development - and some time - since the moieties and their detection systems have not been identified and biochemically characterized for marine species. In the long term, it should be possible to synthesize membrane-bound molecular detectors that function like "artificial noses".

Chemical Attractants

- baits
- peptides by selective enzyme degradation from milk proteins
- empirical trials to identify baits
- control process to determine band width, maybe control diversity of species' attractants
- control specificity
    - prey-specific compounds for predators
    - potential for species-specificity

Summary
A remote technology that is nearer implementation, with some feasibility trials already successfully completed, is the use of various chemical entities as baits and attractants. These may be as simple as peptides produced from milk by selective enzymatic degradation (which are effective on predatory species) to highly specific compounds that target a single species. Although the census would have to be designed appropriately, determination of populations densities and numbers should be possible based on baiting of individuals.

Signature Molecules

- peptides may be species-specific
- high resolution affinity
- lots of water over gel matrix
- use polyclonal antibodies

Summary

Using filtration technologies, large amounts of seawater could be assayed for very low-level concentrations of signature molecules that indicate the presence of particular species or species groups. The detector molecules could be immobilized on gel matrices; remote monitoring of the detector might be feasible.

Self-recognition Systems

- first marine invertebrate pheromones - *Aplysia* albumen gland (Sherry Painter)
- identify organ source
  - can't separate out from seawater
  - need to identify molecule from target species
  - target some species, then develop
- chemical taxonomies of pigments from phytoplankton

Summary

Self-recognition systems are likely sources of signature molecules and highly specific receptor systems. The search for these systems may be facilitated by concentrating on the biochemical systems that organisms use to identify "self" (i.e., conspecifics). These systems are robust and evolutionarily essential: they should thus be stable, precise, and accurate.

Biosensors - olfactory receptors cloned (5 year horizon)

- pheromonal receptors
- spiny lobster (Barry Aki)
- cloned receptor in frog egg (Michael Lemer)
- behavioral assay
- identify receptors
- cloned - choose system
- express receptors on membranes and artificial receptors (fishes)
- pheromonal control - cloning of insect olfactory receptors (John Hildebrand)

Summary

Biosensor development has received a great deal of research attention in recent years. Some of these systems may be close to implementation for marine species, including lobsters. Requirements include cloning of the receptor systems (for immobilization on membranes) and chemical analysis of the signature molecule. The first step is to conduct behavioral studies, to determine whether the artificial biosensor can reliably reproduce the behavioral repertoires of the in vivo system.

Detection Systems

- flourescent tags - for highly specific molecules
- high performance liquid chromatography (HPLC) for diagnosis - probably diagnostic to some groups (gadoids, sharks, lampreys)
- affinity chromatography

<u>Summary</u>
Most detection systems rely on expensive equipment and human manipulation of samples. Although HPLC is labor intensive, it yields highly specific and detailed information on chemical moieties that may in fact by diagnostic. Once a detector molecule is identified and characterized, rapid detection systems (based on fluorescent labels and tags) may be devised for implementation.

Molecular Techniques
(Previously hybridization probes, usually PCR-based)
Future implementations:

- "molecular beacons"
- automated gel reading by image analysis
- gel scans - densitometry
- flourometric markers
- DNA chip technology
- qualitative assay via serial dilution; also presence/absence

<u>Summary</u>
A number of molecular characters are useful as detector molecules, with specificities ranging from an individual, to a species, and an assemblage. Most molecular analysis to date has involved PCR-based methods with gel-based detection, which are probably not suitable for remote systems. We should focus on liquid-phase detection systems, including small molecules that signal detection with fluorometric indicators. Molecular beacons are hybridization probes that recognize variable levels of specificity. The detection system is in a liquid (buffer), where binding separates a fluorescent tag from a quencher sequence, so the "beacon" lights up when the target is detected.

Surface Signatures

- multiplex antibodies/glycoproteins in fluid bath - light up according to group
- nature genetics electric charge
  - cells lysed on plate
  - extracted DNA sticks in wells
  - probe/PCR from there
- needs identification of surface moieties (especially broad taxanomic cuts), then squish and do species-specific analysis

Summary
Remote species' identification will be enormously easier if we identify surface signatures that exhibit the desired level of specificity. Then, it will not be necessary to manipulate or even harm the organism (by homogenization or DNA extraction) in order to identify it. There may be surface glycoproteins or polyclonal antibodies that are species- or group-specific that could serve as indicator molecules.

Detection Systems

- image analysis
- colorimetric system
- flow injection system
    - sorting by successive valves
    - detection by surface proteins
    - laser detection
    - Fluorescent Automatic Cell Sorter (FACS)
- enzyme flourochrome
    - flourescently labeled chitinase as detection test for crustaceans

Summary
A variety of detection systems currently exist that are specific and accurate enough to provide the basis for species' identification. The primary consideration will be expense, ease of use, and suitability for remote application. As a group, we considered a flow-injection system, with a reaction chamber, with a laser-based detection and sorting capacity, to be a conceptual starting place. Similar instruments now exist, such as the Fluorescent Automatic Cell Sorter (FACS) developed by Penny Chisholm (MIT) and Rob Olson (WHOI) to identify phytoplankton cells. Detection systems could be based on the "molecular beacons" (highly specific, fluorescently-labeled oligonucleotid probes) or enzyme fluorochrome assays (in which fluorescently-labeled enzymes light when bound to their specific substrate).

Hierarchical Analysis

Summary
Throughout both the chemical and molecular discussions, we envisioned a hierarchical approach to species' identification, in which the detection of a functional group or assemblage of organisms would trigger the specific detection systems that could appropriately assess the makeup of the group. Not all questions will require quantitative data at the species' level.

Usefulness of Molecular Approaches to Identity Large Marine Organisms

Adult Fish

- non-destructive sampling (mucous, scales, feces)
- sediment traps for fish - marine snow contains fish scales and feces

strong brine as a preservative
time-series with sample rosette
problem of how to back-calculate what sample represents

- "snatching" stations/fish washing station
  chemical/physical
  artificial reefs
- in association with multi-sensor platform - take sample of tissue/blood with microsampler, maybe trap, sample, and release

Marine Mammals

- individual fingerprints

  Summary

  Although we focused on detection of small organisms (using a flow-injection detection system), we also believed that molecular approaches are useful for large organisms. The population geneticists and conservation biologists would consider it a missed opportunity not to collect information allowing the assessment of intraspecific genetic diversity of a limited number of target species, including fish and mammals. In some cases, even large organisms (e.g., gelatinous plankton) cannot be identified reliably by morphological characters. In some case, such as marine mammals, fingerprinting of individuals would provide tags of movement, breeding structure, and reliable identification.

Other Important Points

- population size - levels of genetic diversity
- stock structure/migration
- within species variaton
  very important for ecological, evolutionary, conservation issues
  not possible for remote application
- ancillary data
  pollution
  evolutionary relationships
  genetic diversity within species
  DNA damage
  chemical analysis of water

Summary

The chemical and molecular technologies that are appropriate for identification of species also yield considerable ancillary data that are useful and important. Based on quantification of genetic diversity within a species, inferences can be made about population size, stock structure and migration patterns, ecological and evolutionary characteristics, conservation biology. There are chemical and molecular assays of DNA damage, chemical properties of water, pollution, and ecosystem health. The potential disadvantage of molecular approaches - the need for tissue samples for analysis - is more than compensated for by the wide spectrum of issues addressed by these data and the power of conclusions based on these new technologies.

Consensus System for Chemical/Molecular Sampling of Small Organisms

Flow-Injection Identification and Sorting System (FIISS)

FIISS is a remote, automatable system to detect, identify, sort, and collect small marine organisms. The organisms are drawn into a reaction chamber (by pumping) where indicator molecules are introduced by flow-injection. Possible indicator molecules include hybridization probes, polyclonal antibodies, etc. carried on "beacon" molecules or similar. Detection is by fluorometric or colorimetric systems sensed by lasers. A mixture of target species is identified and sorted, based on a laser-driven automatic shunting system at the excurrent end of the system.

DAY 2

DAY 2 AGENDA

8-9 am	Introduction (Parrish)	Pacific Forum
9-12:30 pm	Super-Predator Breakouts Group 1 Group 2 Group 3	Pacific Forum 1st flr. Break Room 2nd flr. Break Room
12:30-1:30 pm	Lunch	Pacific Forum
1:30-2:30 pm	Plenary/Group Reports & General Summary	Pacific Forum

DAY 2 GROUP TASKS

Following from the Day 1 group reports and armed with the resultant matrices, participants were reassigned to three interdisciplinary groups each tasked with designing a Super-Predator. In addition to the design, groups were asked to identify constraints. both to their design as well as to a RSID program in general. Groups were given the following caveats and guidelines:

Caveats

- Consider a habitat focus along the continua:
- Consider an organism focus from viruses to whales

Guidelines

- Identify the type(s) of sensor(s) used
- How are these sensors integrated?
- Cued - one cues many
- Canalized - cuing sequence is fixed

- All on
- How are data retrieved?
- Data quality - how are identifications groundtruthed? Can all groundtruthing be internal (i.e., among sensory systems) or does some proportion of groundtruthing need to be external (i.e., by a person with the sensor output and/or the organism in hand).
- How many copies should be manufactured?
- What is the estimated expense per copy?
- Demonstration/prototype project - where and how long?

DAY 2 GROUP ASSIGNMENTS

Group 1	Group 2	Group 3
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DAY 2 REPORTS

Group 1
Chair and Rapporteur: Dennis Hedgecock

A Super-Predator must be able to detect and identify a huge size range of marine organisms (viruses to whales issue). No single predator will be able to do this. For smaller organisms (phytoplankton to zooplankton), integrated multisensor systems exist now. An example is BIOMAPER II, which currently possesses acoustics, bio-optical sensors, video plankton recorder (VPR), and a range of enviromental sensors. If the FIISS system was integrated into the VPR, then BIOMAPER would have molecular capabilities. For larger organisms (fish+), another Super-Predator must be designed.
A Super-Predator must recognize both known and new forms. New forms/species may predominate in relatively unexplored places. It is critical that new forms be properly identified as such.

How are new species identified now?

- standard taxonomy (organism in hand)
- molecular systematics (library needed)

How might new species be identified in the near future?

- chemical analysis for pheromones/signature molecules (library needed)

Therefore, new organisms must be caught to be briefly sampled if not kept for human groundtruthing. As we learn more about the organisms in an area, our sensing systems can become "smarter" at recognizing them remotely.

The novel contribution of this group was to design a system which initially integrates humans but becomes smart enough to be "left behind" i.e., remote.

Open Ocean Observatory - O3

O3 is based on an earlier *Deep Sea Observatory* proposal which called for converting an oil drilling platform into a mobile marine laboratory. O3 goes beyond this concept by specifying a timeline for platform deployment and remote sensing spin-up (approximately 2 years per site), followed by a fully remote phase in which the sensory systems left behind continue to record species richness and abundance, as well as ancillary information specific to the needs of the program and the capabilities of the sensors. O3 is the RSID stepping stone.

During the initial deployment phase, O3 acts as a neural net with a central brainstem (the platform) which directs the actions and flightpaths of series of more mobile nodes (AUV, ROV, ABE (autonomous benthic explorer), ships with towed arrays) as well as the sensing systems on any moored arrays with fixed, low frequency acoustics which localize centers of biological activity. Remote and manned vehicles equipped to collect novel biota are deployed. These systems are also equipped with a set of integrated sensors (e.g., BIOMAPER II) such that remotely sensed libraries can be developed and groundtruthed by scientists onboard O3. Because O3 is manned, scientists can also direct the deployment of remote sensing technologies to resample areas of interest. Because O3 is mobile, it can be deployed to remote areas where previous sampling has been inadequate. Once area and technology-specific libraries have be developed and tested, O3 will move to a new location, leaving behind a RSID mooring for time-series multi-sensor data. Costs for initial retrofitting of an oil platform might run 15-10 million; daily operation costs should not be different from a large oceanographic vessel.

Group 2

Chair and Rapporteur: Edie Widder

Super Sensor System for Survey & Synthesis - S5

S5 is a modular multisensor package designed to take advantage of integrated, miniaturized sensors. S5 can be deployed as an ambush (e.g., mooring) or pursuit (e.g., ROV, AUV) predator. In pursuit mode, S5 communicates with ship-board low frequency sonar which directs the initial pursuit path. As an AUV, S5 may dock to download data and repower (e.g., REMUS). In either predator mode, S5 may also communicate with passive listening arrays (e.g., SOSUS). As new sensor systems come online, S5 can evolve to incorporate them, either as add-ons or as replacements for aging technologies.

The novel contribution of this group was to realize that deployment is a non-issue if all sensor systems are modular. In this case, the Super-Predator can evolve from one form to another, as well as through time to incorporate new sensory systems.

S5 Demonstration Project
As Super-Predators such as S5 come online, it is important to create demonstration projects which show how much we don't know about the world's oceans. "What we really want to see is what we've been missing." A demonstration project should be adequate in space (continuous long-scale transect) and time (3 month minimum) to put S5 through a series of sea trials designed to test the Super-Predator under a variety of deployment conditions and bioregions/marine habitats. Essentially, a biological WOCE. Costs for such a project might range 2-3 million for hardware, plus an additional 6-10 million for design, testing (sea trials) and sensor integration.

Group 3
Chair: Tommy Dickey, Rapporteur: John Horne

In order to design a Super-Predator, there are several issues which need to be addressed: objectives, potential platforms, which tools can be used for which taxa, and sampling strategies.

The novel approach of this group was to link the Super-Predator concept to specific sampling strategies rather than to specific machine design.

Objectives
Super-Predators should be designed to:

- quantify richness (i.e., species counting)
- census abundance by taxon (also as a function of size, mobility, and/or habitat)

In pursuit of these objectives, and as a consequence of the types of sensors used, there may be data collected on one or more of the following:

- taxon
- abundance
- location
- time of day/year
- size
- population density (derived from abundance and location data)
- recruitment (derived from abundance and size distribution data)
- health (environmental/organismal)

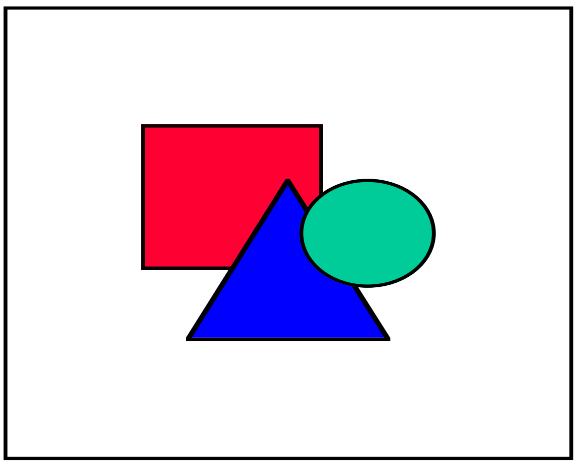
Platforms

- ships (profile/towyoy, of opportunity, underway, with retrievable or expendable sampling packages)
- ROVs
- moorings/arrays (with possible use of attractants)

- drifters (retrievable or expendable)
- marine mammals (sealions, elephant seals, dolphins)
- airborne (remote sensing, deploy expendables)
- satellites (complimentary, sampling guidance, telemetry)
- AUVs (large and sensor intensive, e.g., Autosub; small and with limited sensors, e.g., Odyssey, Remus)
- robo-tuna (mechanical noses which identify a wide variety of chemical exudates, linked to a mobile system whose direction is chosen based on chemical input)
- birds

Sampling Tools

A Super-Predator must detect, identify, and enumerate. Detection and identification requires analysis and interpretation of one or more signals. Tools operate on different scales, and are also useful for different aspects of this three-pronged requirement:



The following table lists tools appropriate for various broad taxonomic categories, grouped principally as a function of size and mobility:

Phytoplankton	**Zooplankton**	**Fish**	**Mammals**
microscope flow cytometry flourescence HPLC OC meters biochemical sensing DNA bioluminescence plane & satellite colorimetry	nets,bottles acoustics video biochemical sensing DNA bioluminescence	nets, traps passive acoustics active acoustics optics telemetry biochemical sensing DNA	passive acoustics active acoustics visual tagging biochemical sensing DNA

Sampling Strategies

Given the wide range of size and mobility of marine organisms to be censused (viruses to whales issue), as well as the challenge of designing a single Super-Predator with all available sensors, we envision Super-Predators as sampling strategy concepts rather than machines per se.

Cascade Sampling Strategy

Use a biochemical attractant to "seed" or catalyze a series of predator-prey interactions. Essentially, create a hotspot or ephemeral community within a defined spatial framework which can be sampled definitively. Both stationary and mobile sensor packages can be deployed to identify and count the organisms assembled.

Dynamic Sampling Strategy

Use coarse-grained sensors (e.g., acoustics) to identify areas of biological activity (hotspots). Further sampling can be targeted in these areas. Robotuna or other biosensors can be used to track down individual species within the hotspot.

WORKSHOP CONCLUSIONS

Sampling and General Identification Issue*s*

- There is a difference between designing a system to enumerate the known and one that also begins to catalog the unknowns. Both things are important. There is also a difference between unknown and undiscovered.

Undiscovered things inhabit places we have undersampled (e.g., deep sea), are destroyed by traditional sampling techniques (e.g., gelatinous zooplankton in nets), or are very small (e.g., viruses and bacteria). Cryptic species result from lumping sister species into a single named taxon.

- We should concentrate on what we don't know, including undiscovered species and undersampled habitats. We should consider concentrated effort in biological hotspots. We should spend more time figuring out where discovered species are in space-time.
- Knowing something about how the physical environment is structured may help us narrow our search effort for biology over a uniform sampling strategy.

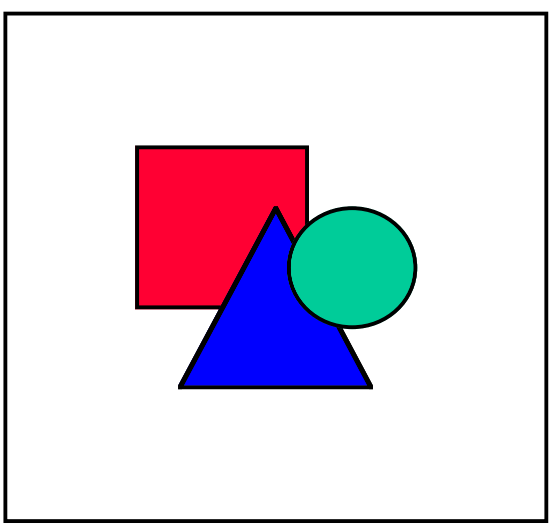
Relevance of Remote Species Identification

- Just enumerating species (i.e., a catalog of species richness) is not a worthy objective; distribution and abundance is a must; ecological (e.g., migration, life history, trophic dynamics, etc.) and environmental measures (e.g., physical habitat, water properties, pollution, etc.) are also important data.

- All sensory systems rely on a reference library for matching/identification. Given a sufficient library, pattern recognition systems - whether acoustic, optical, chemical, or DNA-based - can be trained to detect species remotely.
- Humans are needed to create the libraries needed to train multi-sensor systems and may be needed to perform the ultimate task of decisionmaking re truly new species. This is because the concept of a species is not operationally defined, but rather a subjective decision on the part of the taxonomist. This assertion has two important caveats: First, taxon-specific experts are needed now and into the future. Without this knowledge base, it will not be possible to train Super-Predators, regardless of how clever their detection and identification systems are. Second, the necessity to ultimately have the specimen in hand does not preclude remote species identification. Super-Predators as outlined here may be the only way we can adequately sample habitats such as the deep sea or open ocean within the next few decades.

Design Approach Issues

- Acoustics and optics have dominated species identification and enumeration to date. It is not clear that acoustics alone is sufficient to correctly identify species, especially as the number of species/organisms increases and/or the number of previously undiscovered species increases.
- Super-Predators can use large-scale technologies (e.g., low frequency acoustics) to direct the use of other more fine-scale modalities (e.g., optics). For instance, active acoustics can be used to determine presence/absence and location, while optics and molecular techniques can be used to identify to taxa.
- All technologies are evolving rapidly, making it difficult to predict what types of technologies will be available and to what degree they will accurately identify species remotely, within the next 10 years. The positive side of this uncertainty is that with some re-directed effort currently under-utilized technologies, particularly those centered on



biochemistry, can come on line relatively quickly (i.e., within a 10 year timeframe).

- In general, we should take a lesson from biology and think about how marine organisms perceive both abiotic and biotic signals in the ocean environment.
- Super-Predators should be modular to deal with the issues of deployment, target organism size, and evolving/new technologies.
- Virus-to-whales organism scale variability is a serious issue for Super-Predator design. All organisms can not be dealt with by a single, even multi-sensor system. However, a network of Super-Predators may be able to overcome the scale problem.
- Proximity is a requirement for most types of identification to species (whether remotely or via capture). Stealthy systems (silent, sneaky) are required to get close to many organisms which display aversion to boats, nets, divers, etc. Super-Predators must be behaviorally invisible - they should not provoke unwanted responses, either repulsion or attraction.
- Use of attractants (e.g., light, sound, chemicals) should be seriously considered, perhaps a demonstration project should be developed. We might take a lesson from organisms which use attraction as a predation strategy.
- There may be potential to develop biosensors - blends of organism sensory structure and machine amplifier - to detect taxon-specific signals, especially chemical ones. However, there is a potential ethical dilemma if organisms are harvested for biosensor production.
- Super-Predator design relies on sensory integration and signal processing for internal groundtruthing. These issues need to be explored more fully.

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