



Indicative Sample Analysis and Detailed Sample Analysis

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Sampling Purpose

- Compliance with D-1 (Ballast Water Exchange (BWE))
 - 200 m depth, 200 nm from nearest land
 - 200 m depth, 50 nm from nearest land
 - Designated areas
- Compliance with D-2 (Ballast Water Performance Standard)
 - <10 ind/m³ of orgs. >50 μ m
 - <10 ind/ml of orgs. <50 & $> 10\mu$ m
 - Indicator microbes
 - *Escherichia coli* <250 cfu in 100 ml
 - Enterococci <100 cfu in 100 ml
 - *Vibrio cholerae* <1 in 100 ml or in 1 gr ww zooplankton



Ballast Water Biology

- Almost all types of organisms
- Dominant are crustaceans, molluscs, worms and phytoplankton
- 15 cm long fishes found in tanks
- Harmful algae



**David & Gollasch (eds). Book on
GLOBAL MARITIME
TRANSPORT AND BALLAST
WATER MANAGEMENT –
ISSUES AND SOLUTIONS,
Springer, in prep.**

Two Different Approaches

- Indicative assessment
 - A „quick and dirty“ check for gross exceedence; e.g. 100 orgs = non-compliance

In-depth assessment

- A detailed analysis;
- 10 orgs = non-compliance

Both for

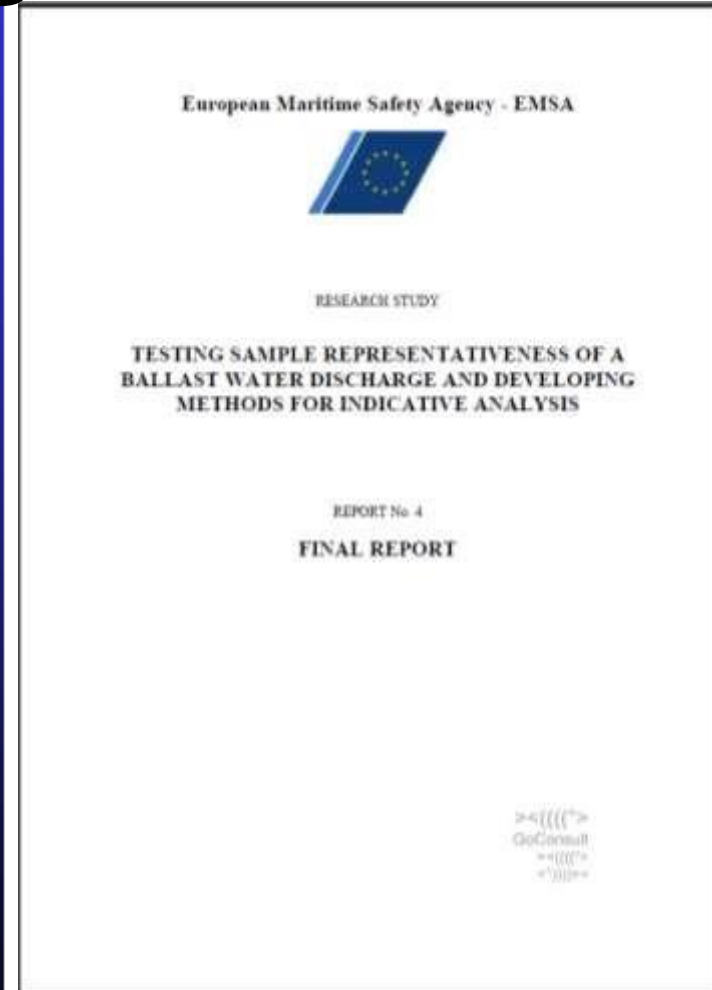
- Sampling and
- Sample analysis



Two Summaries of Organism Detection Methods

EMSA Study
2010

Interreg IV B
Ballast Water
Opportunity
2012

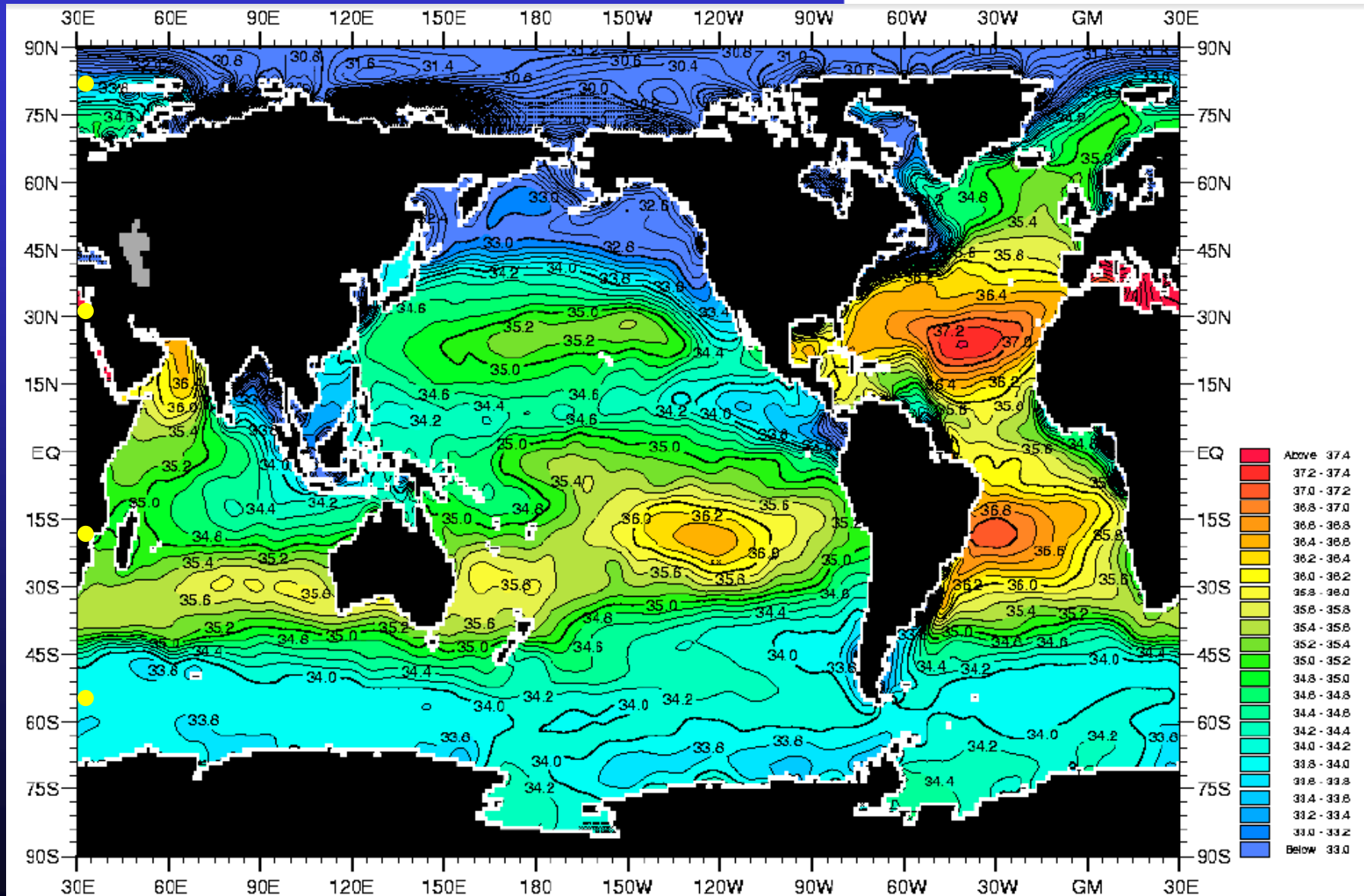


Possible Indicative Methods

- Accuracy
- Reliability
- Time to a result
- Expertise
- Portability
- Costs



D-1 (Ballast Water Exchange)



D-1 (Ballast Water Exchange)

- Salinity
 - If salinity is below 25 psu it is unlikely that it was exchanged at sea
- Tracers of human activity
 - Presence of e.g. Nitrogen or Phosphorous may indicate nearshore BWE (river run-off in urban areas)
- Coastal species
 - Harpacticoid copepods, barnacles
- Sediment
 - High sediment load may indicate near-shore BWE, but re-suspension from tank bottom occurs

Indicative Analysis Methods

D-2 organism groups

- Organisms less than 50 and greater than or equal to 10 micrometres in minimum dimension
8 methods considered

Organisms greater than or equal to 50 micrometres in minimum dimension

- 6 methods considered
- Methods for bacteria analysis
11 methods considered



Indicative Analysis Methods

Organisms less than 50 and greater than or equal to 10 μm in minimum dimension

Presence/absence (no viability, no counts)

e.g. DNA, ATP (new NIOZ method), Chl a methods
deliver results in less than 60 minutes

Viability and counts

Flow cameras or flow cytometry
(< 60 minutes, not portable,
viability stain needed)



Indicative Analysis Methods

Organisms less than 50 and greater than or equal to 10 μm in minimum dimension

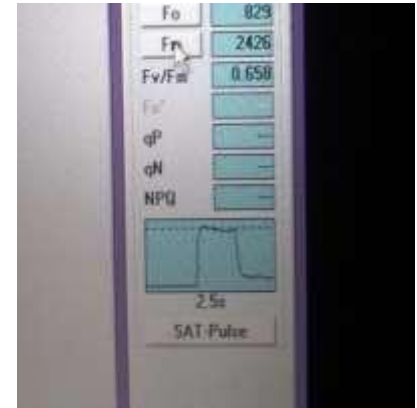
Problem with machine count is cell vs single colony and human error

Best compromise: PAM

portable, easy to use, low expertise needed

Viability in less than 10 minutes

No counts, but biomass and Chl a indication



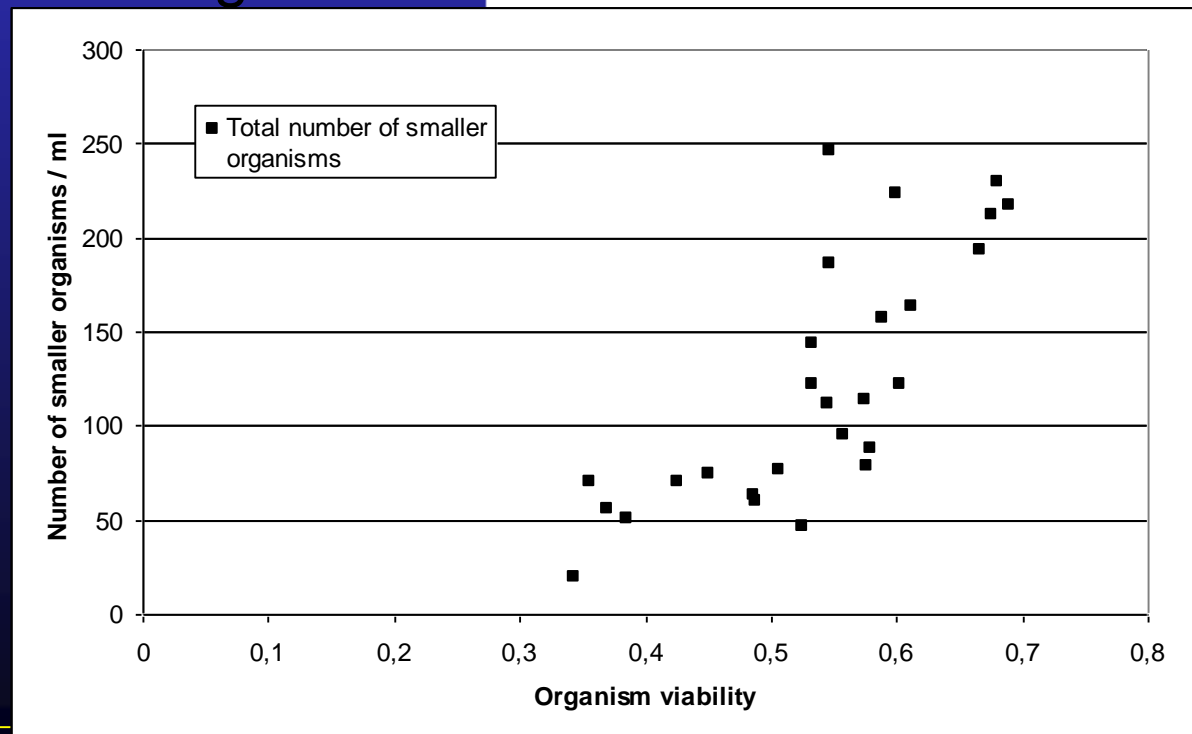
Pulse-Amplitude Modulated fluorometry (PAM)

PAM measures phytoplankton biomass and viability

No direct counts

Clear relation of biomass and viability measurements with organism numbers

Suitable tool to
show clear
grounds that
D-2 was not
Met
Detection limit
is
1 org / ml
(calculated)



New Phytoplankton Methods



→ Ballast Water OK
(Safe to de-ballast)



Ballast Water Bad
(Please see local authorities)

- Thumbs up/Thumbs down

Welschmeyer

Turner

Hach

Bbe



This is now considered a
commercially viable market

Indicative Analysis Methods

Organisms greater than or equal to 50 μm in minimum dimension

Presence/absence methods (no viability, no counts)

e.g. DNA, ATP methods deliver results in less than 60 minutes

Counts (no viability)

Hand-held flow cameras (less than 30 minutes)

Best compromise: Stereomicroscope (counts & viability)

portable, easy to use, high expertise needed

results in less than 20 minutes

Indicative Analysis Methods

D-2 Bacteria

Presence/absence methods (no cfu and/or counts)

e.g. DNA, ATP methods deliver results in less than 60 minutes

All methods to determine cfu require incubation time of 24 - 72 hours

Best compromise: Hand-held fluorometer

portable, easy to use, low expertise needed

presence/absence in < 10 mins to 4 hours

semiquantitative, i.e. high reading = high bacteria numbers

Indicative Analysis Methods, Suggestions 1

Start with one method to evaluate one organism group in D-2

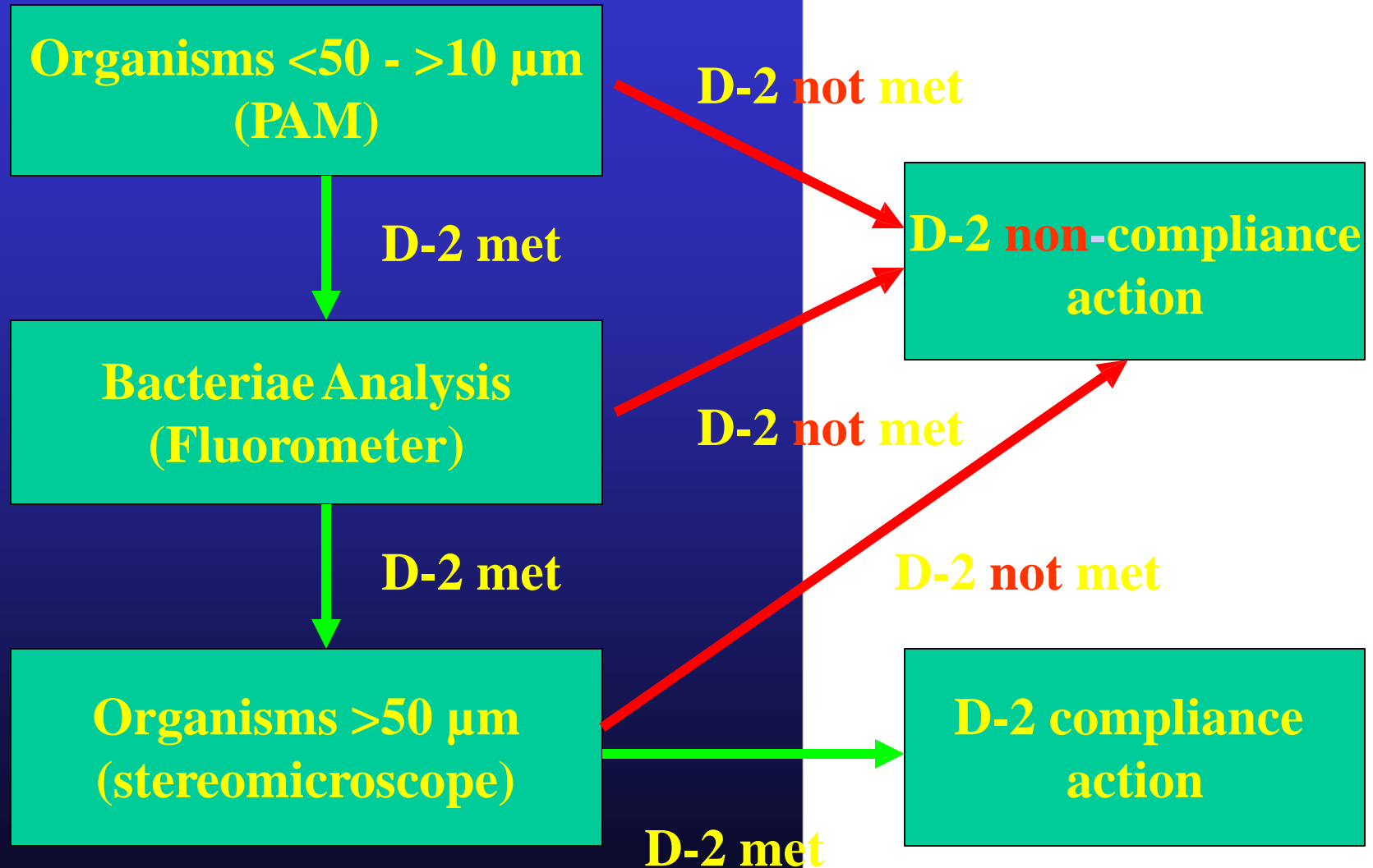
Should this show presence or high numbers, take result as indication of a failed treatment system

Should this show absence or low numbers, continue with second (and third) D-2 organism group to confirm results

The easiest to start with may be the analysis for phytoplankton (Pulse-Amplitude Modulated fluorometry , PAM), followed by bacteriae (hand-held fluorometer) and zooplankton (stereomicroscope)

To evaluate if a ballast water treatment system using active substances (chemicals) was operated remaining active substances may be measured

Indicative Analysis Approach



Indicative Analysis Methods, More Suggestions

Consider to equip a van with organism detection technology

Drive from vessel to vessel in a port

Send sampling team onboard and deliver the samples as soon as possible to van for analysis

In this scenario the organism detection tools would not need to be carried onboard

↗ Sampling team “only” to board the vessel, no need to bring organism detection team onboard as well

Detailed Analysis

- Work according to good laboratory practice standards
- Most accurate analysis technologies should be used
- At best consider living samples
- Keep time between sample taking and sample analysis as short as possible
- Unlikely to have a standard world-wide or in Europe. Instead work towards standards set by an Administration?

Detailed Analysis Methods

D-2 organism

Organisms less than 50 microns and greater than 10 microns in dimension

Microscopy, bright-field epifluorescens



Sedgwick-Rafter
Cell



Detailed Analysis Methods

D-2 organisms greater than or equal to 50 micrometres in minimum dimension



Detailed Analysis Methods

D-2 organisms greater than or equal to 50 micrometres in minimum dimension

- **Microscopic analysis**
- **Stains don't work 100%**
- **Living dead judgement by a scientist**



Detailed Analysis Methods

D-2 methods for bacteria analysis

- colony forming units to identify
- not all bacteria form colonies
- incubation needed
- selective media



Detailed Analysis Methods

D-2 methods for bacteria analysis



Analysis Methods, Summary

No single method to address all D-2 organism Groups

1). Presence/absence methods

**Document presence of organisms, but no counts, neither viability analysis
Suitable as first indication**

2) Count and viability methods

**Document number of viable organisms
Suitable for D-2 compliance control**

Conclusions

- Methods exist to proof D-1 & D-2 compliance
- Compromise needed considering what can be done when working on a vessel (number of samples, volumes)
- Harmonized approach, not that one vessel is compliant in one port, but not in another
- An indicative sample analysis may be followed by a detailed analysis of the same sample
- What to do in case of proven non-compliance? No ballast water discharge!

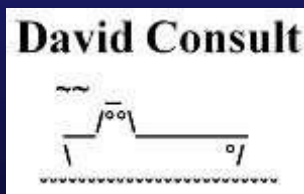
**Thank you very much
for your attention**



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